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AMINOCARBOXYLATE LIGANDS HAVING SUBSTITUTED AROMATIC AMIDE MOIETIES

This is a continuation-in-part of copending
5 U.S. Application Serial No. 07/738,998, filed August
1, 1991.

Background of the Invention

Metal-chelating ligands are useful in
10 diagnostic medicine as contrast agents. X-ray
imaging, radionuclide imaging, ultrasound imaging and
magnetic resonance imaging can each be enhanced by
the use of a metal atom bound to a chelating ligand.
For example, a chelating ligand can become a
15 radiopharmaceutical when it is prepared as a chelate
complex with ^{99m}Tc, ^{111}In, ^{67}Ga, ^{140}La, ^{169}Yb, ^{68}Ga,
^{90}Y, ^{188}Re, ^{153}Sm or other radioactive metal ions.
When a chelating ligand is complexed with the stable
isotopes of the lanthanides, tantalum, bismuth or
20 other elements with molecular weight higher than
iodine, the resulting complex absorbs x-rays
sufficiently to act as an x-ray contrast agent. In
some cases, the agents that are useful in x-ray
imaging absorb, reflect or scatter ultrasound
25 radiation sufficiently to be used as an ultrasound
agent. If a chelating ligand is complexed with a
paramagnetic metal atom that has a symmetric
electronic ground state (e.g., Gd^{+3}, and octahedral
Mn^{+2}, Fe^{+3}, Cr^{+3}) the resulting complex will be useful

as a spin relaxation catalyst that is used in magnetic resonance imaging (also known as NMR imaging) as a contrast agent. If a chelating agent is complexed with a paramagnetic metal atom that has

5 an unsymmetrical electronic ground state (e.g., dysprosium(III), holmium(III) and erbium(III), the resulting complex will be useful as a chemical shift agent in magnetic resonance imaging or in magnetic resonance _in vivo_ spectroscopy. In addition, any

10 paramagnetic metal ion complex may be used as a contrast agent by virtue of its magnetic susceptibility as disclosed by Villringer et al. (_Magnetic Resonance in Medicine_, 6, 164-174, 1988).

The chelating ligands can also be

15 bifunctional. That is, they can bind tightly to the metal ion forming a chelate while at the same time bearing a second functionality which confers upon it desirable chemical, physical and/or biological properties. Desirable physical properties of the

20 chelator differ depending on the diagnostic or therapeutic purpose of the metal chelate. Desirable physical properties common to all uses are high affinity for the metal ion bound to the chelator, and ease of synthesis. When it is desired to use the

25 metal chelate as a contrast medium for NMR imaging or general purpose x-ray imaging, the desirable physical properties are high water solubility, high chemical stability and viscosity and osmolality of a formulated drug solution as close as possible to

30 those of human blood. Further, in the specific instance of a spin relaxation catalyst, the greatest possible relaxivity is desired. Relaxivity as used herein is understood to be as the effectiveness, per

mole of complex, of altering the relaxation times of
the nuclei being imaged.

Human blood has an osmolality of 0.3 Osmol/kg-
water. Hyperosmolality is a well known contributor
5   to adverse patient reactions to injected contrast
media, and the lower osmolality of newer x-ray agents
is due to their being nonionic molecules (possessing
a net zero overall charge) (Shehadi, W. H.; "Contrast
media adverse reactions: occurrence, reoccurrence and
10   distribution patterns", Radiol, 1982, 143, 11-17.
Bettman, M. A.; "Angiographic contrast agents;
conventional and new media compared", Am. J.Roentgen,
1982, 139, 787-794. Bettman, M. A. and Morris, T. W.;
Recent advances in contrast agents, Radiol. Clin.
15   North Am., 1986, 24, 347-357.). Many gadolinium-
based NMR agents in the prior art that are useful
have a net negative overall charge, and therefore
their aqueous formulated solutions have high
osmolality. For example, $Gd(DTPA)^{2-}$ where DTPA stands
20   for diethylenetriaminepentaacetic acid is formulated
for use at 0.5M in water as the N-methylglucamine
salt. The osmolality of the solution is 1.6 to 2.0
Osmol/kg-water. New nonionic Gd complexes are
described in U. S. 4,859,451 and 4,687,659. The
25   preferred new gadolinium complexes of the present
invention are nonionic - they are not salts. When
these nonionic gadolinium complexes are formulated at
0.5M in water the osmolality of the solutions is 0.3-
0.6 Osmol/kg-water. The complex should be generally
30   inert to interaction with the body other than general
tissue distribution and excretion, usually by the
renal route, without, or minimally, depositing Gd
metal in tissues for long periods of time. Gd
complexes of macrocyclic aminocarboxylates are

generally more chemically inert than Gd complexes of linear aminocarboxylates (P. Wedeking and M. Tweedle, <u>Nucl. Med. Biol.</u>, 15, 395-402, 1988; M. Tweedle et al., <u>Magn. Reson. Imog.</u>, 9, 409-415, 1991; and M.

5   Tweedle, "Contrast and Contrast Agents in Magnetic Resonance Imaging", edited by P. A. Rink, <u>European Workshop on Magnetic Resonance in Medicine</u>, 1989) The preferred aminocarboxylate ligands for Gd are therefore members of the macrocyclic aminocarboxylate

10 class, and are, in addition, nonionic.   These properties are important to NMR imaging, but, in addition, the effectiveness of an agent for NMR imaging can be increased by altering the chemical structure so as to increase the ability of the metal

15 chelate to affect the relaxation times of water protons.

In radiopharmaceutical imaging the doses administered are relatively small so that matching the drug formulation's physical properties to those

20 of human blood is relatively unimportant.   In this use biological specificity is more important.   In particular, one could use ^{99m}Tc as the metal and a chelating ligand which is functionalized with a biologically active entity such as a bile acid, fatty

25 acid, amino acid, peptide, protein or one of numerous chemical entities known to bind receptors <u>in vivo</u>. NMR contrast media may also make use of biological specificity.

In radiopharmaceutical therapy, the metal ions

30 may be chosen from among those known in the art; for example, ^{90}Y, ^{188}Re, ^{153}Sm.   For this purpose the chelating ligand is generally covalently bound to a disease specific entity such as monoclonal antibody. When the metal-chelator-antibody conjugate is

injected into humans, it concentrates at the disease site, usually a malignant tumor. In this use the chelating ligand must contain a reactive functionality which allows for a covalent bond to be

5　formed between the chelating ligand and the antibody. Important characteristics of the reactive functionality are as follows: (1) it must be covalently attached to the chelator such that it does not significantly diminish the affinity of the

10　chelator for the metal ion; (2) it must allow simple synthesis in high yield of metal-chelator-antibody conjugates, the conjugate so-formed should have maximal affinity for its antigen, such affinity being minimally diminished as a result of covalently

15　attaching the metal-chelator; (3) it should ideally allow for rapid excretion and/or optimal dosimetry of the radioactive metal chelator in the event that the metal-chelator-antibody conjugate is decomposed or metabolized _in vivo_.

20　　　When the metal is non-radioactive and paramagnetic such as gadolinium (III), the bifunctional chelate is useful in magnetic resonance imaging as a contrast agent, either as a discrete molecule or bound to substances such as lipids,

25　sugars, alcohols, bile acids, fatty acids, receptor-binding ligands, amino acids, peptides, polypeptides, proteins, and monoclonal antibodies. When the metal is radioactive, such as yttrium(III) as ^{90}Y, the bifunctional chelate is useful in labeling monoclonal

30　antibodies for use in radiotherapy. When the metal is ^{99m}Tc, ^{111}In, ^{201}Tl, ^{67}Ga, ^{68}Ga or the like, the chelate is useful in radiopharmaceutical imaging.

　　　Two general methods have been employed for making bifunctional chelates from chelating agents.

In the first method one or more carboxylic acid groups of a polyaminopolycarboxylic acid chelator are activated by conversion to such activating groups as internal or mixed anhydrides, activated esters (e.g.,

5   p-nitro phenyl, N-hydroxysuccinimide, etc.) or with other derivatives known to those skilled in the art. The activated acid group is then reacted with the protein. The metal ion is then added to the protein-chelator complex.

10   There are two problems with this method. First, using a potential donor group, the carboxylic acid, to react with the protein can diminish the strength of the chelate and contribute to the chemical lability of the metal ion. The second

15   problem arises because the chelating ligands have several carboxylates that are not uniquely reactive. When the chelating ligand is combined with an activating agent more than one species can result because the number and chemical position of the

20   groups activated cannot be adequately controlled. When a mixture of such variously activated chelating ligands is added to protein, protein-chelator complexes of variable and uncertain chelating strength can be formed. Also, multiple activation of

25   carboxylic acids on a chelator leads to intra- and inter-molecular crosslinking which is a major source of decreased immunospecificity. This problem could be overcome by separating all of the products formed from the reaction of the activating agent with the

30   chelating ligand, but that process is very laborious and makes the overall synthesis highly inefficient.

         The second method for making a bifunctional chelate is to prepare a chelating ligand with a unique reactive function, such as an isothiocyanate,

attached to the chelating ligand at a position that does not substantially diminish the strength with which the chelating ligand binds the metal ion. An article entitled "Synthesis of 1-(p-isothiocyanato-

5 benzyl) derivatives of DTPA and EDTA, Antibody Labeling and Tumor-Imaging Studies" by Martin W. Brechbiel, Otto A. Gansow, Robert W. Atcher, Jeffrey Schlom, Jose Esteban, Diane E. Simpson, David Colcher, Inorganic Chemistry, 1986, 25, 2772 is

10 illustrative of the above second method. Also, U. S. Patent 4,885,363 describes these methods as they apply specifically to nonionic macrocyclic aminocarboxylates.

Wedeking et al., "Biodistribution and

15 Excretion of New Gd-Complexes in Mice", Abstracts of the 8th Annual Meeting of the Society of Magnetic Resonance in Medicine, 801, 1989, have disclosed the compound
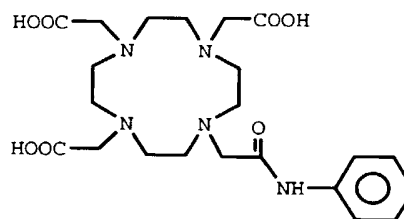
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When used to chelate a paramagnetic ion, e.g., Gd, in magnetic resonance imaging, this compound was found to have poor water solubility, although acceptable

25 relaxivity.

Brief Description of the Invention

It is an object of this invention to provide new metal-chelating ligands.

It is an object of this invention to provide new metal chelate complexes that are nonionic.

Another object is to provide metal chelating ligands which when complexed with a metal heavier

5 than iodine (e.g., Ba, Ta, Pb, Bi, Lanthanides) are effective as x-ray contrast agents.

Another object is to provide metal chelating ligands which when complexed with gamma emitting radioactive nuclide (e.g., ^{99m}Tc or ^{111}In) are

10 effective as imaging radiopharmaceuticals.

Another object is to provide metal chelating ligands which when complexed with beta or alpha emitting radioactive nuclide (e.g., ^{90}Y, ^{153}Sm, ^{188}Re, ^{212}Bi) are effective as therapeutic

15 radiopharmaceuticals.

It is a further object of this invention to provide metal-chelating ligands whose metal chelate complexes in aqueous solution have low osmolality.

It is a further object of this invention to

20 provide metal-chelating ligands whose metal chelate complexes have low acute toxicity.

It is a further object of this invention to provide metal-chelating ligands which, when complexed with a paramagnetic metal atom, are effective as

25 relaxation catalysts in magnetic resonance imaging.

It is a further object of this invention to provide bifunctional metal-chelating ligands that have the ability to covalently bind to proteins or other biologically active molecules thereby imparting

30 biological specificity to the metal chelate complex. The conversion of the novel molecules described herein to bifunctional chelates is accomplished using the methods described above.

It is a further object of this invention to provide new metal complexes with increased relaxivity.

It is a further object of this invention to
5 provide bifunctional metal-chelating ligands that are thermodynamically stable, kinetically inert and, when desired, electrically neutral.

These, and other objects which will be appreciated by the practitioner of this invention,
10 are achieved by substituting at one of the nitrogen atoms of an aminocarboxylate ligand a substituted aromatic amide moiety of the formula

I

$$-(CH_2)_m-\overset{\overset{\displaystyle O}{\|}}{C}-\overset{\overset{\displaystyle R_{13}}{|}}{N}-A_1-\underset{R_2}{\overset{R_1}{\bigcirc}}$$
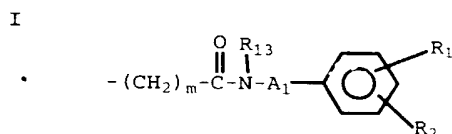
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wherein

A_1 is $-(CH_2)_{m'}-$ or a single bond;

$(CH_2)_m$ and $(CH_2)_{m'}$ may independently be substituted with alkyl or hydroxyalkyl;

20 R_{13} is hydrogen, alkyl, arylalkyl, aryl, alkoxy, hydroxyalkyl;

R_1 and R_2 are each independently hydrogen, alkyl, $-NO_2$, $-NH_2$, $-\overset{\overset{\displaystyle S}{\|}}{NHCNHR_{12}}$, NCS, $-\overset{\overset{\displaystyle O}{\|}}{C}-NR_3R_4$, NR_3COR_9, where R_9 is alkyl or hydroxyalkyl, with the proviso
25 that at least one of R_1 and R_2 must be other than hydrogen;

R_3 and R_4 are independently hydrogen, alkyl, arylalkyl, aryl, alkoxy and hydroxyalkyl;

R_{12} is hydrogen, alkyl or hydroxyalkyl;
30 m and m' are independently 1 to 5;

and multimeric forms thereof.

Preferred are those compounds where A_1 is a single bond.
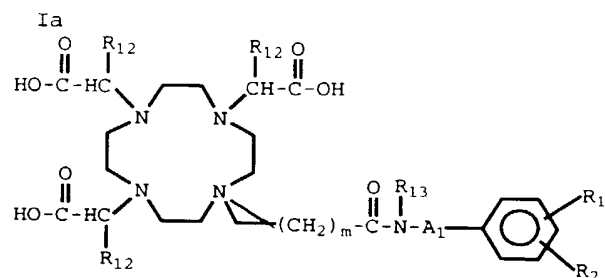
Detailed Description of the Invention

The terms "alkyl" and "alkoxy" as used
throughout the specification, refer to both straight
and branched chain groups. Those groups having 1 to
5 carbon atoms are preferred and methyl is the most
preferred alkyl group.

The term "aryl" as used throughout the
specification refers to phenyl and substituted
phenyl. Preferred substituted phenyl groups are
those substituted with 1, 2 or 3 halogen, hydroxyl,
hydroxyalkyl, alkyl, alkoxy, carbamoyl, carboxamide,
acylamino or carboxyl groups.

Hydroxyalkyl refers to straight and branched
alkyl bearing radicals R-OH groups such as $-CH_2CH_2OH$,
$-CH_2CHOHCH_2OH$, $CH(CH_2OH)_2$ and the like. Such
chemistry is well known to those skilled in the art
(Sovak, M., editor, Radiocontrast Agents, Springer-
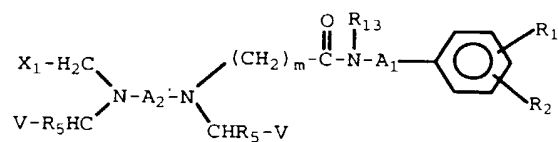Verlag, 1984, pp. 1-125).

As described above, aminocarboxylate nuclei
known in the art can be provided with a substituted
aromatic amide moiety of formula I to provide the
novel compounds of the present invention.

Exemplary novel aminocarboxylates having a
substituted aromatic amide moiety include compounds
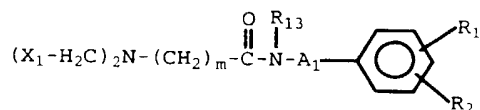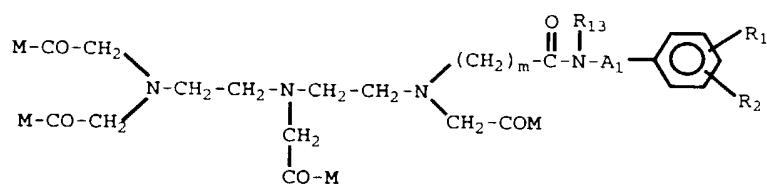of the formula

Ia

$$HO-\overset{\overset{O}{\|}}{C}-\overset{\overset{R_{12}}{|}}{HC}\quad N\cdots\cdots N\quad \overset{\overset{R_{12}}{|}}{CH}-\overset{\overset{O}{\|}}{C}-OH$$

$$HO-\overset{\overset{O}{\|}}{C}-\overset{\overset{|}{HC}}{\underset{R_{12}}{}}\quad N\cdots\cdots N-(CH_2)_m-\overset{\overset{O}{\|}}{C}-\overset{\overset{R_{13}}{|}}{N}-A_1\text{—}\langle\text{ring}\rangle\overset{R_1}{\underset{R_2}{}}$$

5

Ib

$$\begin{array}{l} X_1-H_2C \\ \quad\quad\quad\quad N-A_2-N \\ V-R_5HC \end{array}\begin{array}{l} (CH_2)_m-\overset{\overset{O}{\|}}{C}-\overset{\overset{R_{13}}{|}}{N}-A_1\text{—}\langle\text{ring}\rangle\overset{R_1}{\underset{R_2}{}} \\ \\ CHR_5-V \end{array}$$

Ic

$$(X_1-H_2C)_2N-(CH_2)_m-\overset{\overset{O}{\|}}{C}-\overset{\overset{R_{13}}{|}}{N}-A_1\text{—}\langle\text{ring}\rangle\overset{R_1}{\underset{R_2}{}}$$

10

Id

$$\begin{array}{l} M-CO-CH_2 \\ \quad\quad\quad\quad\quad N-CH_2-CH_2-N-CH_2-CH_2-N \\ M-CO-CH_2 \end{array}\begin{array}{l} (CH_2)_m-\overset{\overset{O}{\|}}{C}-\overset{\overset{R_{13}}{|}}{N}-A_1\text{—}\langle\text{ring}\rangle\overset{R_1}{\underset{R_2}{}} \\ \\ CH_2-COM \end{array}$$

with CH_2 and $CO-M$ on the central nitrogen

15

wherein in formulae Ia, Ib, Ic and Id, m, R_{13}, A_1, R_1, R_2 and R_{12} are as defined above for formula I and further wherein

X_1 is $-COOY_1$, PO_3HY_1 or $-CONHOY_1$;

Y_1 is a hydrogen atom, a metal ion equivalent and/or a physiologically biocompatible cation of an inorganic or organic base or amino acid;

A_2 is $-CHR_6-CHR_7-$, $-CH_2CH_2(ZCH_2-CH_2)_n-$,

$$-CH_2-\overset{\overset{\displaystyle N(CH_2X_1)_2}{|}}{CH}-CH_2 \quad \text{or} \quad -CH_2-CH_2-\overset{\overset{\displaystyle CH_2-CH_2-N(CH_2X_1)_2}{|}}{N}-CH_2-CH_2- \quad , \text{ wherein}$$

X_1 is as defined above;

each R_5 is hydrogen or methyl;

R_6 and R_7 together represent a trimethylene group or a tetramethylene group or individually are hydrogen atoms, lower alkyl groups (e.g., 1-8 carbons), phenyl groups, benzyl groups or R_6 is a hydrogen atom and R_7 is $-(CH_2)_p-C_6H_4-W-$protein where p is 0 or 1, W is $-NH-$, $-NHCOCH_2-$ or $-NHCS-$, protein represents a protein residue;

n is 1, 2 or 3;

Z is an oxygen atom or a sulfur atom or the group NCH_2X_1 or $NCH_2CH_2OR_8$ wherein X_1 is as defined above and R_8 is C_{1-8}alkyl;

V is X_1 or is $-CH_2OH$, $-CONH(CH_2)_rX_1$ or $-COB$, wherein X_1 is as defined above, B is a protein or lipid residue, r is an integer from 1 to 12, or if R_5, R_6 and R_7 are each hydrogen; then both V's together form the group

$$-(CH_2)_w-\overset{\overset{\displaystyle CH_2X_1}{|}}{N}-CH_2-CH_2-\overset{\overset{\displaystyle CH_2X_1}{|}}{N}-(CH_2)_w-$$

where X_1 is as above, w is 1, 2 or 3, provided that at least two of the substituents Y_1 represent metal ion equivalents of an element with an atomic number of 21 to 29, 42, 44 or 57 to 83; from 1 to 4, advantageously 2 or 3, and preferably 3 M's are $-OH$ and the balance independently are $-OR_{10}$, $-NH_2$, $-NHR_{10}$

and/or $NR_{10}R_{10}'$ wherein R_{10} and R_{10}' are selected from an organic alkyl radical of up to 18 carbon atoms which may be substituted.

The compounds of formulae Ia, Ib, Ic and Id
5 and salts thereof, can be complexed with a para-magnetic metal atom and used as relaxation enhance-ment agents for magnetic resonance imaging. These agents, when administered to a mammalian host (e.g., humans) distribute in various concentrations to
10 different tissues, and catalyze relaxation of protons (in the tissues) that have been excited by the absorption of radiofrequency energy from a magnetic resonance imager. This acceleration of the rate of relaxation of the excited protons provides for an
15 image of different contrast when the host is scanned with a magnetic resonance imager. The magnetic resonance imager is used to record images at various times generally before and after administration of the agents, and the differences in the images created
20 by the agents' presence in tissues are used in diagnosis. In proton magnetic resonance imaging, paramagnetic metal atoms such as gadolinium(III), and octahedral manganese(II), chromium(III) and iron(III) (all are paramagnetic metal atoms with a symmetrical
25 electronic configuration) are preferred as metals complexed by the ligands of formula I; gadolinium(III) is most preferred due to the fact that it has the highest paramagnetism, low toxicity, when complexed to a suitable ligand, and high
30 lability of coordinated water.

The metal-chelating ligands of the present invention can be complexed with a lanthanide (atomic number 58 to 71) and used as chemical shift agents in

magnetic resonance imaging or in magnetic resonance _in vivo_ spectroscopy.

While the above-described uses for the metal-chelating ligands of the present invention are preferred, those working in the diagnostic arts will appreciate that the ligands can also be complexed with the appropriate metals and used as contrast agents in x-ray imaging, radionuclide imaging and ultrasound imaging.

Use in Imaging

To use the ligands of this invention for imaging, they must first be complexed with the appropriate metal. This can be accomplished by methodology known in the art. For example, the metal can be added to water in the form of an oxide or in the form of a halide or acetate and treated with an equimolar amount of a ligand of the present invention. The ligand can be added as an aqueous solution or suspension. Dilute acid or base can be added (if needed) to maintain a neutral pH. Heating at temperatures as high as 100°C for periods up to four hours is sometimes required, depending on the metal and the chelator, and their concentrations.

Pharmaceutically acceptable salts of the metal complexes of the ligands of this invention are also useful as imaging agents. They can be prepared by using a base (e.g., an alkali metal hydroxide, meglumine or arginine) to neutralize the above-prepared metal complexes while they are still in solution. Some of the metal complexes are formally uncharged and do not need cations as counterions. Such neutral complexes are preferred as intravenously administered x-ray and NMR imaging agents over charged complexes because they provide solutions of

greater physiologic tolerance due to their lower osmolality.

Sterile aqueous solutions of the chelate complexes can be administered to mammals (e.g., humans) orally, intrathecally and especially intravenously in concentrations of 0.003 to 1.0 molar. For example, for the visualization of brain lesions in canines using magnetic resonance imaging, a gadolinium complex of a ligand of formula I can be adminstered intravenously at a dose of 0.05 to 0.5 millimoles of the complex per kilogram of animal body weight, preferably at a dose of 0.1 to 0.3 millimoles/kilogram. For visualization of the kidneys, the dose is preferably 0.05 to 0.25 millimoles/kilogram. For visualization of the heart, the dose is preferably 0.25 to 1.0 millimoles/kilogram. The pH of the formulation will be between about 6.0 and 8.0, preferably between about 6.5 and 7.5. Physiologically acceptable buffers (e.g., tris-(hydroxymethyl)aminomethane) and other physiologically acceptable additives (e.g., stabilizers such as parabens) can be present.

It is also advantageous to employ dual scavenging excipients such as those described in a copending application U. S. Ser No. 682,487 filed April 9, 1991 entitled "DUAL FUNCTIONING EXCIPIENT FOR METAL CHELATE CONTRAST AGENTS". Those excipients have the general formula

$$X_m[X'(L')]_n$$

wherein X and X' are independently Ca or Zn, L' is an organic ligand which may be different than or the

same as the ligand employed to complex the metal and m and n are independently 1, 2 or 3.

Use of Radiotherapy or Imaging Where the Metal-Chelate-Complex is Bound to a Biomolecule

The bifunctional metal-chelating ligands can bind to a monoclonal antibody or a fragment thereof for use in radiotherapy. Monoclonal antibodies are useful in that they can be used to target radionuclides to cancer or tumor sites with great specificity. The compounds of this invention wherein R_1 is other than hydrogen are then linked to monoclonal antibodies or fragments thereof.

The methods of linking the bifunctional chelate to the antibody or antibody fragment are known in the art (Brechbiel, same reference as referred to hereinabove) and will depend primarily on the particular bifunctional chelate and secondarily on the antibody or fragment thereof. For example, when the formula Ia compound is R_1 = H, R_2 = -NCS or

$$-NHCNHR_{12},$$ one reacts 10 μL of a 5.0 mM aqueous solution of the formula I chelator with 0.5 mL of a 5.0 mg/mL monoclonal antibody (B72.3 purchaseable from Damon Biotech Corporation) in 50 mM Hepes buffer at pH 8.5. 16 μL of 1.5M aqueous triethylamine is added. After 2 hours reaction time, the monoclonal antibody is purified by dialysis. This procedure provides between 1 and 2 formula I chelator molecules bound to each monoclonal antibody. Radioactive metal ion (for example ^{90}Y) can then be added to the monoclonal antibody-bound chelator by methods known in the art. For example, ^{90}Y as the $^{90}Y(III)(acetate)_3(H_2O)_4$ (approximate formula in

aqueous solution) can be reacted with the monoclonal antibody-bound chelate in solutions where the concentration of each is between 10^{-5} and 10^{-7}M and the pH is 6. Dialysis against citrate is then used to
5   purify the product.

An alternative, and preferred method follows that described above, but substitutes the metal-chelate complex for the chelating ligand. To use this method the metal chelate complex is first made
10   by reacting metal-oxide, -halide, -nitrate, -acetate, or the like with formula I chelator. For the chelator described above the acetate of ^{90}Y at $<10^{-6}$M is reacted with the chelator at about 10^{-3} at pH 6, the chelate complex is purified by ion exchange or
15   reverse phase HPLC choromatography, and then reacted with the monoclonal antibody described above for the chelator. The bifunctional, metal-containing, linked antibody is used in the following manner. A human or animal with a tumor to which the monoclonal antibody
20   is specific is injected intravenously, subcutaneously, intraperitoneally or intralymphatically for example, with an aqueous solution of the ^{90}Y-formula I chelator-monoclonal antibody compound. This allows the radioactive metal ion to be directed to the tumor
25   for which it is intended. The intravenous dosage used is 0.1 to 0.4 millicuries per kilogram of body weight.

Preferred embodiments for when the compounds are linked to a protein are when R_1 and/or R_2 = NCS
30   is reacted with protein to produce the protein conjugate. Preferred proteins are those in serum, wherein the R_1 and/or R_2 = -NCS compound is directly injected.

It is understood that other functional groups known in the art can be used to link the bifunctional metal-chelating ligands of this invention to monoclonal antibodies or fragments thereof.

5   R_1 and R_2 are each $-\overset{\overset{\displaystyle O}{\|}}{C}-NR_3R_4$ and R_3 in each is hydroxyalkyl in a preferred embodiment for forming a Gd(III) chelate useful in general purpose magnetic resonance imaging. The most preferred embodiments for forming a Gd(III) chelate are when the R_3 groups

10 are each $-CH_2-\underset{\underset{\displaystyle OH}{|}}{CH}-CH_2OH$ or $-CH(CH_2OH)_2$, especially $-CH_2-\underset{\underset{\displaystyle OH}{|}}{CH}-CH_2OH$, and the R_4 groups are each hydrogen.

The present invention also includes multimeric forms of the compounds of formula I, such as dimers, trimers, tetramers, etc. Known functional groups and

15 technology as those discussed above regarding conjugation with biomolecules are readily useable to provide such multimers. The functional groups provided onto the phenyl ring
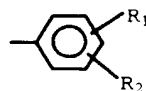
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can be, for example, R_2 = NCS or $-NH\overset{\overset{\displaystyle S}{\|}}{C}NHR_{12}$, especially where R_{12} is methyl or ethyl. Thus, exemplary multimers of formula I

25   I'
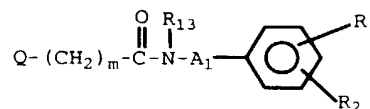
where Q is the aminocarboxylate nucleus of Ia, Ib, Ic or Id, are shown by

<u>Dimers</u>
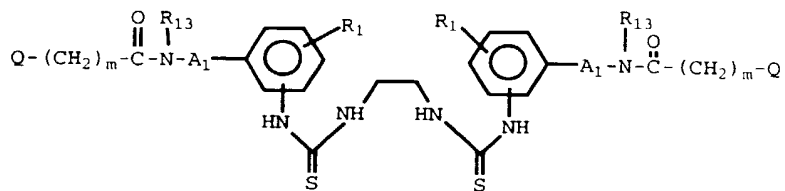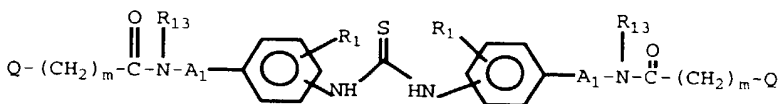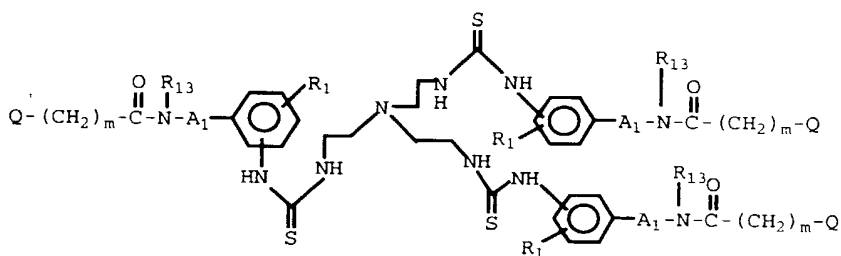
$$Q-(CH_2)_m-\overset{\overset{\displaystyle O}{\|}}{C}-\overset{\overset{\displaystyle R_{13}}{|}}{N}-A_1-\underset{\underset{\displaystyle HN}{}}{\bigcirc}^{R_1} \quad \overset{R_1}{\underset{\underset{\displaystyle NH}{}}{\bigcirc}}-A_1-\overset{\overset{\displaystyle R_{13}}{|}}{N}-\overset{\overset{\displaystyle O}{\|}}{C}-(CH_2)_m-Q$$

$$\underset{\displaystyle S}{\overset{\displaystyle \|}{C}}-NH-CH_2CH_2-NH-\underset{\displaystyle S}{\overset{\displaystyle \|}{C}}$$

or

$$Q-(CH_2)_m-\overset{\overset{\displaystyle O}{\|}}{C}-\overset{\overset{\displaystyle R_{13}}{|}}{N}-A_1-\underset{\underset{\displaystyle NH}{}}{\bigcirc}^{R_1} \quad \overset{\overset{\displaystyle S}{\|}}{C} \quad \overset{R_1}{\underset{\underset{\displaystyle HN}{}}{\bigcirc}}-A_1-\overset{\overset{\displaystyle R_{13}}{|}}{N}-\overset{\overset{\displaystyle O}{\|}}{C}-(CH_2)_m-Q$$

<u>Trimers</u>

$$Q-(CH_2)_m-\overset{\overset{\displaystyle O}{\|}}{C}-\overset{\overset{\displaystyle R_{13}}{|}}{N}-A_1-\bigcirc^{R_1}$$

Hexamers

$$X-N(H)-CH_2CH_2-N< ...$$

and the like,

where X =

$$\underset{\text{S}}{\overset{\|}{C}}CH_3-NH-\text{(ring)}-A_1-N(R_1)-\overset{O}{\overset{\|}{C}}-(CH_2)_m-Q$$

Preparation of Formulae Ia, Ib, Ic and Id Compounds

To prepare the compounds of formula Ia, a compound of the formula

IIa

$$HO-\overset{O}{\overset{\|}{C}}-CH(R_{12}) ... CH(R_{12})-\overset{O}{\overset{\|}{C}}-OH$$
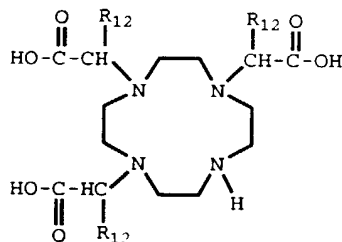
is reacted in a solvent, e.g., water, and in the presence of a base, e.g., sodium hydroxide, with a compound of the formula

III

$$L-(CH_2)_m-\overset{O}{\overset{\|}{C}}-N(R_{13})-A_1-\text{(ring)}<\overset{R_1}{\underset{R_2}{}}$$
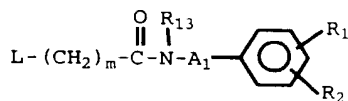
wherein L is a leaving group, such as halogen. The
preparation of compounds of formula II is well known,
for example, in U. S. 4,885,363 to Tweedle et al.
For example, in preparing compounds of formula II,
5 reaction of a compound of the formula

IV

$$H-N \underset{\underset{CH_2}{\overset{CH_2}{|}}}{\overset{CH_2-CH_2}{\diagdown}} N-H$$

where Y is $\diagup N CHO$
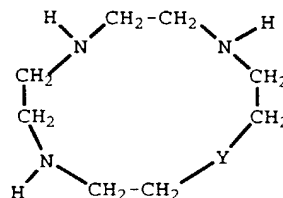
10· with a compound of the formula

V

$$L-\underset{R_{12}}{\overset{R_{12}}{\underset{|}{CH}}}-\overset{O}{\overset{||}{C}}-OH$$
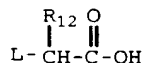
wherein L is a leaving group such as halogen
15 is preferably carried out in water at a pH of about
8.5 to 11 and the temperature of the reaction is
maintained at about 45°-55°C. Preferably, only about
two equivalents of a compound of formula V are
initially used in the reaction; an additional
20 equivalent of the compound of formula V is added in
portions starting about 2 to 3 hours after ¨ e
reaction begins. Total reaction time will preferably
be about 8 to 24 hours. The desired trisubstituted
product can be separated from the reaction mixture,
25 which includes the mono-, di-, tri- and tetra-
substituted derivatives, by techniques recognized in
the art including selective precipitation,
chromatography and crystallization.

A preferred preparation of the compounds of formula IIa wherein R_{12} is hydrogen is to react 1,4,7,10-tetraazacyclododecane, known in the art, with dimethylformamidedimethylacetal in the presence

5 of benzene to yield 1,4,7,10-tetraazatricyclo-[5.5.1.0]tridecane. This "tricyclic" compound is reacted with an ethanol/water mixture to yield 1-formyl-1,4,7,10-tetraazacyclododecane. This formyl compound is then reacted with t-butyl bromoacetate to

10 yield 1-formyl-4,7,10-triscarboxymethyl-1,4,7,10-tetraazacyclododecane, tris-t-butylester. Finally, the ester groups are removed in the presence of strong acid, such as sulfuric acid, to yield a compound of formula IIa wherein R_{12} is hydrogen. The

15 most preferred methods are included in Dischino, et al., _Inorg. Chem._, _30_, 1265, 1991.

Compounds of formula III wherein R_1 and R_2 are each $-\overset{\displaystyle O}{\overset{\displaystyle \|}{C}}NHR_3$ and R_{13} is hydrogen are prepared by first reacting a compound of the formula

20 VI
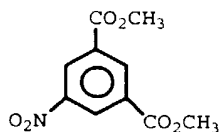
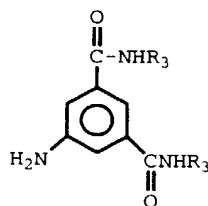in a solvent, e.g., methanol, with a compound of the formula

VII

25 H_2NR_3

to provide the intermediate

VIII

$$\underset{O_2N}{\overset{\overset{O}{\overset{\|}{C}}-NHR_3}{\bigcirc}}\overset{C-NHR_3}{\underset{\|}{O}}$$
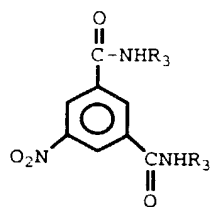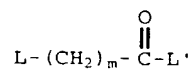
Compounds of formula VIII can thereafter be
reduced, e.g., with hydrogen in the presence of a
palladium on carbon catalyst, to provide

IX

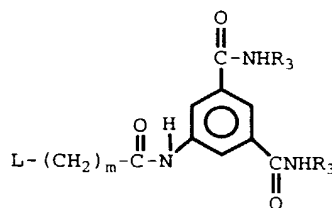$$\underset{H_2N}{\overset{\overset{O}{\overset{\|}{C}}-NHR_3}{\bigcirc}}\overset{C-NHR_3}{\underset{\|}{O}}$$

Reaction of compound IX with a compound of the
formula

X

$$L-(CH_2)_m-\overset{O}{\overset{\|}{C}}-L'$$

wherein L and L' are the same or different leaving
groups, e.g., halogens, in a solvent, e.g., dimethyl-
acetamide, provides the compounds of the formula

III'

$$L-(CH_2)_m-\overset{O}{\overset{\|}{C}}-\overset{H}{\overset{\|}{N}}\overset{\overset{O}{\overset{\|}{C}}-NHR_3}{\bigcirc}\overset{C-NHR_3}{\underset{\|}{O}}$$

that is, compounds of formula III where R_1 and R_2 are

each $-\overset{\overset{O}{\|}}{C}NHR_3$, A_1 is a single bond, and R_{13} is
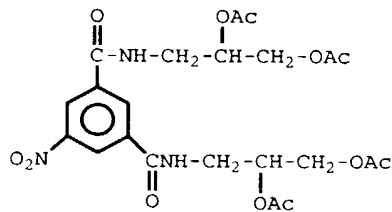
hydrogen.

In the event that the R_3 group in intermediate VIII contains hydroxyalkyl moieties, the hydroxy groups are converted to acetyloxy groups after the reaction of compounds VI and VII to obtain VIII'. For example, if the compound of formula VII is

VII'

$$H_2N-CH_2-\overset{\overset{OH}{|}}{CH}-CH_2-OH$$
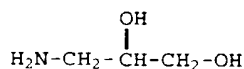
reaction as described above with compound VI provides

VIII'

,

wherein Ac is acetyl. Following reduction to the corresponding aniline and thereafter reaction with compound X, the corresponding intermediates of III' i.e., wherein R_3 is acetyloxy alkyl, are converted to their hydroxyalkyl counterparts by known treatment, e.g., with sodium methoxide in a solvent, for example, methanol.

Compounds of formula III wherein A_1 is $-CH_2-$, R_{13} is as defined above, L is chloro and R_1 and R_2 are
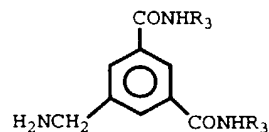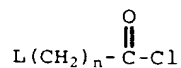
each $\overset{\overset{\displaystyle O}{\displaystyle \|}}{-C}NHR_3$ are prepared by first reacting a compound
of the formula

VIII

CONHR$_3$

O$_2$N   CONHR$_3$

with gaseous hydrogen in the presence of a catalyst
such as palladium on carbon in dilute mineral acid to
provide the aniline

IX

CONHR$_3$

H$_2$N   CONHR$_3$

The aniline is diazotized with nitrous acid in
acidic medium and then treated with sodium cyanide to
obtain

XI

CONHR$_3$

NC   CONHR$_3$.

Reduction of the nitrile XI in the presence of
a platinum catalyst with gaseous hydrogen at low
pressure, for example 3 atmospheres, affords

XII

CONHR$_3$

H$_2$NCH$_2$ CONHR$_3$.

Reaction of XII with a compound of formula
XIII

$$L(CH_2)_n-\overset{\overset{\textstyle O}{\|}}{C}-Cl$$

wherein L is a leaving group, for example chlorine,
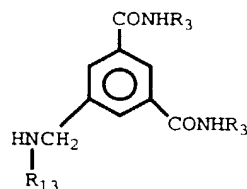provides compounds of the formula

III·
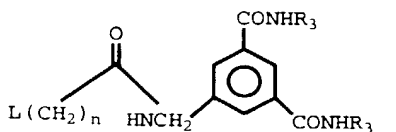
If R_{13} is other than hydrogen, for example
methyl, the compound of the formula XII is treated
with an aldehyde R_8CHO, for example formaldehyde,
wherein R_8 is H, under reducing conditions, for
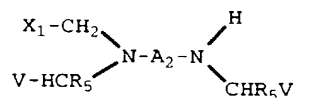example with sodium borohydride, to obtain

IX·

wherein R_{13} is methyl. Reaction of IX· with the
chloride of formula XIII will provide the desired
intermediate of the formula

III"'

$$L(CH_2)n \quad \underset{\underset{R_{13}}{|}}{NHCH_2} \quad \text{(benzene ring with } CONHR_3 \text{ and } CONHR_3)$$
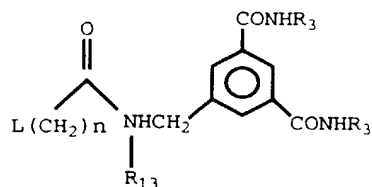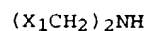
Similarly, compounds of formulae Ib, Ic and Id
can be prepared by reacting the various compounds of
formula III in a solvent, e.g. water, and in the
presence of a base, e.g., sodium hydroxide with the
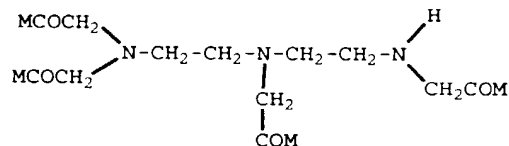corresponding compounds

IIb

$$\underset{V-HCR_5}{\overset{X_1-CH_2}{\diagdown}} N-A_2-N \overset{H}{\underset{CHR_5V}{\diagup}} \quad ,$$

IIc

$$(X_1CH_2)_2NH$$

or

IId

$$\underset{MCOCH_2}{\overset{MCOCH_2}{\diagdown}} N-CH_2-CH_2-\underset{\underset{\underset{COM}{|}}{CH_2}}{N}-CH_2-CH_2-N \overset{H}{\underset{CH_2COM}{\diagup}}$$

Compounds of formula IIb and IIc are described
in U. S. Patent 4,647,447. Compounds of formula IId
are described in U. S. 4,859,451.

The invention will now be further described by
the following examples, but is not limited to the
details therein.

Example 1

10-[2-[[3,5-Bis[[(2,3-dihydroxypropyl)amino]-
carbonyl]phenyl]amino]-2-oxoethyl]-1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid,
monogadolinium complex

A.   N,N'-Bis[2,3-bis(acetyloxy)propyl]-5-nitro-
      1,3-benzenedicarboxamide

To a solution of dimethyl-5-nitroisophthalate
(23.9 g, 100 mmol) in methanol (300 mL) was added
1-amino-2,3-propanediol (20.2 g) and the mixture was
refluxed for 48 hours. Methanol was removed in
vacuo, the residue was dissolved in pyridine (150 mL)
and then treated with acetic anhydride (80 mL) at
room temperature for 16 hours. Excess acetic
anhydride was decomposed by adding water (50 mL) to
the reaction mixture. The solvents were removed in
vacuo, the residue dissolved in ethyl acetate (400
mL) and washed with water (2 x 100 mL), 10%
hydrochloric acid (200 mL) and finally with brine
(100 mL). The ethyl acetate layer was dried and
removal of the solvent afforded the title A nitro-
bis-amide (51.8 g), as a light yellow viscous syrupy
material. This was used directly without further
purification in the next step.

B.   5-Amino-N,N'-bis[2,3-bis(acetyloxy)propyl]-
      1,3-benzenedicarboxamide

A solution of the title A nitrobisamide (31.5
g, 60 mmol) in methanol (180 mL) was hydrogenated
over 10% palladium on carbon (300 mg) for a period of
3 hours. The catalyst was filtered off and the
solvent removed in vacuo to afford pure title B

aniline (28.6 g), as a viscous syrupy material. This
was used directly without further purification in the
next step.

5 C.   N,N'-Bis[2,3-bis(acetyloxy)propyl]-5-N-
            [(chloroacetyl)amino]-1,3-benzenedicarboxamide
          The title B aniline was dissolved in dimethyl-
acetamide (150 mL) and treated with chloroacetyl
chloride (11.28 g, 100 mmol) dropwise over a period
10 of 20 minutes. The solution was stirred for 3 hours
and dimethylacetamide removed in vacuo. The residue
that resulted was dissolved in ethyl acetate, washed
with water, 10% aqueous sodium bicarbonate, and
finally with water. The ethyl acetate layer was
15 dried and removal of the solvent afforded the crude
chloroacetanilide (32.0 g). The crude material was
purified by column chromatography over silica gel to
obtain the title C compound (26.3 g), as a colorless
glassy solid. An analytical sample was prepared by
20 crystallizing 1.00 g of the glassy solid from ethyl
acetate/hexane. m.p. _____ (68°-72°)
Elemental Analysis calc'd for $C_{24}H_{30}N_3ClO_{11}$:
            C, 50.40; H, 5.29; N, 7.35; Cl, 6.20;
            O, 30.77%.
25 Found: C, 50.28; H, 5.15; N, 7.11; Cl, 6.25%.

D.   5-[(Chloroacetyl)amino-N,N'-bis[2,3-
            dihydroxypropyl]-1,3-benzenedicarboxamide
          A solution of the title C compound (25.6 g, 45
30 mmol) in methanol (200 mL) was treated with
sodium methoxide (20 mmol) and the solution was
stirred at 0° for 30 minutes. The pH of the reaction
mixture was adjusted to 7 by adding Dowex 50 (H$^+$)
resin, the resin filtered off and the methanol

removed _in vacuo_ to afford pure title D compound as a
colorless glassy solid (16.8 g). This material was
directly used in the next step without further
purification.

E.   10-[2-[[3,5-Bis[[(2,3-dihydroxypropyl)amino]-
carbonyl]phenyl]amino]2-oxoethyl]1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid

A solution of DO3A sulfate (DO3A = 1,4,7,-
triscarboxymethyl-1,4,7,10-tetraazacyclododecane
prepared in U. S. 4,885,363 to Tweedle et al.) (12.0
g, 27 mmol) was made in water (80 mL) and the pH of
the solution was adjusted to 9.8 by adding 5 M sodium
hydroxide. While maintaining the pH of the solution
at 9.8, a solution of the title D compound (16.4 g,
40.6 mmol) in water (50 mL) was slowly added to the
DO3A solution at 80° over a period of 45 minutes. At
the end of 17 hours, the reaction mixture was cooled
to room temperature, the pH lowered to 3.5 by adding
1 N hydrochloric acid and the solution was desalted
by cation exchange chromatography. Further
purification by anion exchange chromatography
afforded the title E compound as the triethylammonium
salt (19.9 g). The triethylamine salt was dissolved
(6.00 g) in water (1 L), applied to an anion exchange
column and then eluted with 50 mM formic acid to
obtain the desired title E compound (4.9 g). IR:
3400 (OH); 3115 (NH); 1631 (COOH and ArCONH) cm^{-1}.
Mass Spectrum: 714 (M+H)$^+$; 712 (M-H)$^-$.
Elemental Analysis calc'd for $C_{30}H_{47}N_7O_{13} \cdot 0.38H_2O$:
          C, 50.01; H, 6.68; N, 13.61; O, 29.71%.
Found: C, 49.91; H, 6.97; N, 13.42; H_2O, 0.95.%.

F.   10-[2-[[3,5-Bis[[(2,3-dihydroxypropyl)amino]-
      carbonyl]phenyl]amino]-2-oxoethyl]-1,4,7,10-
      tetraazacyclododecane-1,4,7-triacetic acid,
      <u>monogadolinium salt</u>

5   To a solution of the title E triethylammonium
salt (19.00 g, 18.7 mmol) in water (80 mL) at pH 4.72
was added a solution of Gd(OAc)$_3$ · 4H$_2$O (9.83 g, 24
mmol) in water (80 mL) and the reaction mixture was
stirred at room temperature for 12 hours. The
10   reaction mixture was subjected to low pressure
reversed phase column chromatography over a styrene-
divinylbenzene copolymer resin to obtain the title
compound as a colorless glassy solid (17.5 g). The
pure product (17.00 g) was crystallized from hot
15   methanol to afford this title F compound as colorless
needles of >99.9% purity. This sample was
redissolved in water (200 mL), the solvent removed,
and the sample dried in vacuo (1 mm) for four days at
80°. Mass Spectrum: 869 (M+H)$^+$, 867 (M-H)$^{-1}$.
20   Elemental Analysis: calc'd for C$_{30}$H$_{44}$N$_7$O$_{13}$ · 0.36 H$_2$O:
          C, 41.20; H, 5.15; N, 11.21; O, 24.45%.
      Found: C, 40.96; H, 5.07; N, 10.93; H$_2$O, 0.75.%.

<u>Example 2</u>

25

10-[2-[[3,5-Bis-[[[2-hydroxy-1-(hydroxymethyl)ethyl]-
amino]carbonyl]phenylamino]2-oxoethyl]1,4,7,10-
<u>tetraazacyclododecane-1,4,7-triacetic acid</u>

30 A.   N,N'-bis[2-(Acetyloxy)-1-[(acetyloxy)methyl]-
         <u>ethyl]-5-nitro-1,3-benzenedicarboxamide</u>
         To a solution of dimethyl-5-nitroisophthalate
(14.0 g, 58 mmol) in methanol (150 mL) was added
2-amino-1,3-propanediol (16.5 g, 181 mmol) and the

mixture was refluxed for 48 hours. The reaction
mixture was cooled to room temperature and the
crystalline solid that separated out filtered and
dried to obtain the bis amide (19.5 g). A solution

5   of the bis-amide (19.0 g) in pyridine (75 mL) was
treated with acetic anhydride (40 mL) at room
temperature for 16 hours. Excess acetic anhydride
was decomposed by adding water (50 mL) to the
reaction mixture. The solvents were removed in

10 vacuo, the residue dissolved in ethyl acetate, and
the solution washed with water, 10% hydrochloric acid
and finally with brine. The ethyl acetate layer was
dried and removal of the solvent afforded pure title
A nitrobisamide (23.6 g) as a colorless solid, after

15 crystallization from acetone and hexane, m.p. 105-
107°C.

B.   5-Amino-N,N'-bis[2-(acetyloxy)-1-[(acetyloxy)-
      methyl]ethyl]-1,3-benzenedicarboxamide

20   A solution of the title A compound (18.0 g, 34
mmol) in methanol (180 mL) was hydrogenated over
palladium on carbon (0.5 g) for a period of 3 hours.
The catalyst was filtered off and the solvent removed
in vacuo to afford pure title B aniline (16.6 g)

25 after crystallization from acetone and hexane, m.p.
152-154°C.
Elemental Analysis calc'd for $C_{24}H_{30}N_3ClO_{11}$:
       C, 50.40; H, 5.29; N, 7.35; Cl, 6.20%.
Found: C, 50.64; H, 5.20; N, 7.22; Cl, 6.57.%.

30

C.   N,N'-Bis[2-(acetyloxy)-1-[(acetyloxy)methyl]-
      ethyl]-5-(chloroacetyl)amino-1,3-benzene-
      dicarboxamide

The title B compound (17.0 g, 34 mmol) was dissolved in dimethylacetamide (150 mL) and treated with chloroacetyl chloride (7.52 g, 64 mmol) dropwise over a period of 20 minutes. The solution was
5 stirred for 3 hours and dimethylacetamide was then removed _in vacuo_. The residue that resulted was dissolved in ethyl acetate, washed with water, saturated aqueous sodium bicarbonate solution, and finally with water. The ethyl acetate layer was
10 dried and the solvent removed to obtain the crude chloroacetanilide (18.5 g). This material was crystallized from ethyl acetate and hexane to afford pure title C compound (16.8 g), m.p. 135-137°C. Elemental Analysis calc'd for $C_{24}H_{30}N_3ClO_{11}$:
15   C, 50.40; H, 5.29; N, 7.35; Cl, 6.20;
         O, 30.77%.
Found: C, 50.64; H, 5.20; N, 7.22; Cl, 6.57%.

D.   5-[(Chloroacetyl)amino]-N,N'-bis[2-hydroxy-1-
20   (hydroxymethyl)ethyl]-1,3-benzenedicarboxamide
         A solution of the title C compound (16.0 g, 28 mmol) in methanol (200 mL) was treated with sodium methoxide (10 mmol) and the solution stirred at 0° for 30 minutes. The precipitated solid was filtered
25 and dried to afford pure title D compound as a colorless glassy solid (10.8 g), m.p. 222-224°. Mass Spectrum: m/z 404 $(M+H)^+$.

Elemental analysis calc'd for $C_{16}H_{22}N_3ClO_7$:

   C, 47.59; H, 5.49; N, 10.41; Cl,8.78;

   O, 27.73%.

Found: C, 47.66; H, 5.55; N, 9.98; Cl, 8.88%.

E.   10-[2-[[3,5-Bis-[[[2-hydroxy-1-(hydroxy-
      methyl)ethyl]amino]carbonyl]phenylamino]2-
      oxoethyl]-1,4,7,10-tetraazacyclododecane-
      1,4,7-triacetic acid

A solution of DO3A sulfate (6.0 g, 13.5 mmol)
was made in water and the pH of the solution was
adjusted to 9.8 by adding 5 M sodium hydroxide.
While maintaining the pH of the solution at 9.8,
solid N,N'-bis[2-hydroxy-1-[(hydroxy)methyl]ethyl]-5-
N-(chloroacetyl)aminobenzene-1,3-dicarboxamide (8.2
g, 20.4 mmol) was added in small portions to the DO3A
solution at 80° over a period of 45 minutes. At the
end of 20 hours, the reaction mixture was cooled to
room temperature, the pH lowered to 3.5 by adding 1 N
hydrochloric acid and the solution was desalted by
cation exchange column chromatography. Further
purification by anion exchange column chromatography
afforded the title E compound as the corresponding
triethylammonium salt (5.2 g). The triethylammonium
salt (5.2 g) was dissolved in water (1 L) and applied
to an anion exchange column and eluted with 50 m℃
formic acid to obtain the pure title E compound (4.4
g), as a colorless glassy solid.

Elemental analysis calc'd for $C_{30}H_{47}N_7O_{13}$:

   C, 50.48; H, 6.64; N, 13.74; O, 29.14%.

Found: C, 50.34; H, 6.83; N, 13.54%.

F.   The gadolinium chelate of title E compound

The Gd complex of this ligand was prepared by
the same method used for the compound in Example 1.
Elemental Analysis for $C_{30}H_{44}N_7O_{13}Gd$ 3.48 H_2O:

C, 38.72; H, 5.52; N, 10.53; O, 28.33%.

5 Found: C, 39.01; H, 5.37; N, 10.26%.

Example 3

10-[2-[[3,5-Bis[[(2-methylbutyl)amino]carbonyl]-

10 phenyl]amino]2-oxoethyl]1,4,7,10-tetraazacyclo-
dodecane-1,4,7-triacetic acid, monogadolinium complex

A. N,N'-Bis[(2-methylbutyl)amino]-5-nitro-1,3-
benzenedicarboxamide

15 To a solution of dimethyl-5-nitro-isophthalate
(14.0 g, 50 mmol) in methanol was added 2-methyl-
butylamine (12.5 g, 150 mmol) and the mixture was
refluxed for 48 hours. Methanol was removed _in_
vacuo, the residue dissolved in ethyl acetate and

20 washed with 10% hydrochloric acid, 10% aqueous sodium
bicarbonte solution and finally with water. The
ethyl acetate layer was dried and removal of the
solvent afforded the desired compound. This was
crystallized from ethyl acetate and hexane to afford

25 the the title A compound as colorless needles (19.6
g), m.p. 147-148°C.

Elemental Analysis for C, 61.87; H, 7.79; N, 12.03;
O, 18.31%

Found: C, 61.84, H, 7.76; N, 12.02%.

30

B. 5-Amino-N,N'-bis[(2-methylbutyl)amino]-1,3-
benzenedicarboxamide

A solution of the title A compound (17.45 g,
50 mmol) in methanol (180 mL) was hydrogenated over

10% palladium on carbon (500 mg) for a period of 3 hours. The catalyst was filterd off and the solvent removed <u>in vacuo</u> to afford the title aniline as a colorless solid. This was crystallized from acetone and hexane to afford the title B compound as colorless needles (15.8 g), m.p. 170-172°C.

Elemental Analysis for C, 67.87; H, 9:15; N, 13.15; O, 12.02%

Found: C, 68.07, H, 9.30; N, 13.26%.

C.   5-[(Chloroacetyl)amino]-N,N-bis[(2-methyl-butyl)amino]1,3-benzenedicarboxamide

A solution of the title B compound (11.48 g, 36 mmol) in dimethylacetamide (200 mL) was treated with chloroacetyl chloride (5.6 g, 50 mmol) dropwise over a period of 20 minutes. The solution was stirred for 3 hours and dimethylacetamide removed <u>in vacuo</u>. The residue that resulted was dissolved in ethyl acetate (200 mL), washed with water (100 mL), 10% aqueous sodium bicarboante (100 mL) and finally with water (100 mL). The ethyl acetate layer was dried and removal of the solvent afforded the crude chloroacetanilide (12.8 g). This was crystallized from ethyl acetate and hexane to afford the title C compound as colorless needles (11.2 g), m.p. 160-162°C.

Elemental Analysis for C, 60.67; H, 7.64; N, 10.61; Cl, 8.95; O, 12.12%

Found: C, 61.03, H, 7.69; N, 10.57; Cl, 9.22%.

D.   10-[2-[[3,5-Bis[[(2-methylbutyl)amino)-carbonyl]phenyl]amino]2-oxoethyl]1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid

A solution of DO3A sulfate (6.0 g, 13.5 mmol) was made in water (100 mL) and the pH of the solution was adjusted to 9.8 by adding 5 M sodium hydroxide. While maintaining the pH of the solution at 9.8, a solution of the chloroacetanilide (8.2 g, 27 mmol) in ethanol (100 mL) was slowly added to the DO3A solution at 80° over a period of 1 hour. At the end of 17 hours, the reaction mixture was cooled to room temperature, the pH lowered to 3.5 by adding 1N hydrochloric acid and the solution was desalted by cation exchange chromatography. Further purification by anion exchange chromatography afforded the title compound as the triethyl ammonium salt (2.8 g). The triethyl ammonium salt was dissolved in water, applied to an anion exchange column and then eluted with 50 mM formic acid to obtain the desired compound (AAA-DO3A) (2.2 g). A small amount of an impurity present in this sample was further removed by a reverse phase CHP-20 column chromatography to afford the title compound as a colorless glassy solid (1.8 g). Mass Spectrum: 706 $(M+H)^+$; 704 $(M-H)^-$.
Elemental analysis calc'd for $C_{34}H_{55}N_7O_9 \cdot 1.7\ H_2O$:

C, 55.45; H, 7.99; N, 13.31; O, 23.24;
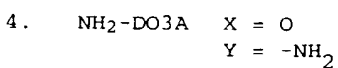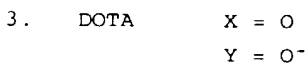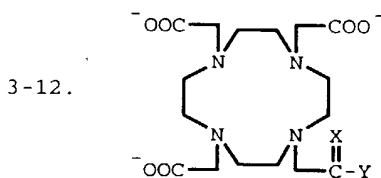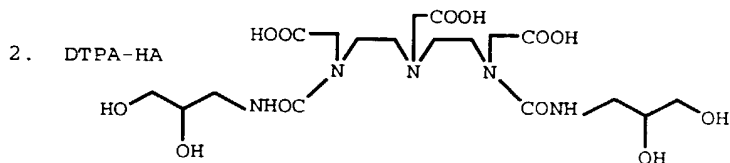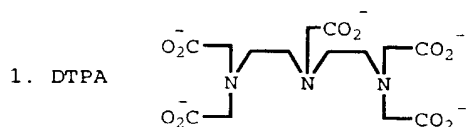Found: C, 55.85; H, 8.37; N, 13.19; H_2O: 4.15.

E.   Gadolinium chelate of title D compound.

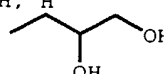The Gd complex of this ligand was prepared by the same method as used for the compound of Example 1.
Elemental anal. calc'd for $C_{34}H_{52}N_7O_9Gd$, 6.44 H_2O:

C, 41.84; H, 6.70; N, 10.04; O, 26.31%.
Found: C, 41.80; H, 6.65; N, 10.28%.

The T_1 relaxivity was measured for nine prior
art gadolinium complexes (1-9) as compared to novel
gadolinium complexes using the ligands of Examples 1,
2 and 3 (#10, 11 and 12, respectively, in the Table
5   below). Relaxivity was measured on an IBM Minispec
spin analyzer operating at 20 MHz and 39±1°C.
Aqueous solutions were used in 0.1-5 mM Gd
concentration range.

10

Structures of Ligands for Gd Complexes of Table 1

1. DTPA

2. DTPA-HA

5

3-12.

3.   DOTA   X = O

10   Y = O⁻

4.   NH₂-DO3A   X = O
                 Y = -NH₂

15 5.   MA-DO3A   X = O
                 Y = -NHCH₃

6.   HEA-DO3A   X = O
                 Y = -NHCH₂CH₂OH

20

7.   PA-DO3A   X = O
                 Y = -NHPhenyl
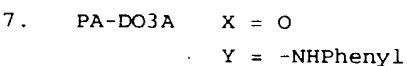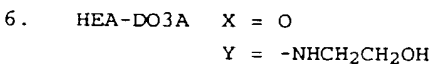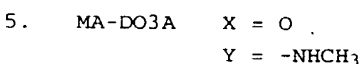
8.   HP-DO3A   X = OH, H
                 Y = -CH₃

9.   PG-DO3A   X = OH, H
                 Y = (CH₃CH₂CH(OH)CH₂OH)

10. HAA-DO3A   X = O
     (Ex. 1)

Y =   -NH—[phenyl ring with 1,3-di(CONH-CH₂-CH(OH)-CH₂-OH) substituents]

11. HAS-DO3A   X = O
     (Ex. 2)

Y =   -NH—[phenyl ring with 1,3-di(CONH-CH(OH)-CH₂-OH) substituents]

12. AAA-DO3A   X = O
     (Ex. 3)

Y =   -NH—[phenyl ring with 1,3-di(CONHCH₂CH(CH₃)-CH₂CH₃) substituents]
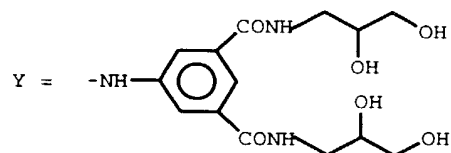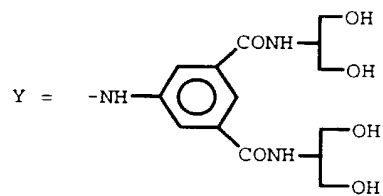
Table 1

Data on Water Soluble Gd Complexes and Ions
Demonstrating The Enhancement of Relaxivity by
N-Hydroxy-alkyl or N-alkyl-isophthalamide Groups and
by Aryl Groups or by Hydroxyalkyl or Alkylamido
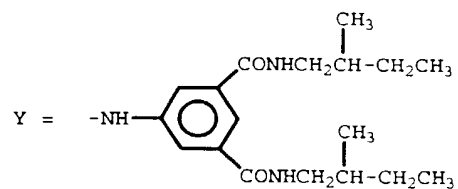Groups.

	Gd(L), L =	T_1 Relaxivity
1.	DTPA	3.7
2.	DTPA-HA	4.4
3.	DOTA	3.4
4.	NH_2-DO3A	3.6
5.	MA-DO3A	4.3
6.	HEA-DO3A	4.3
7.	PA-DO3A	4.1
8.	HP-DO3A	3.7
9.	PG-DO3A	3.4
10.	HAA-DO3A (Ex. 1)	5.8
11.	HAS-DO3A (Ex. 2)	5.4
12.	AAA-DO3A (Ex. 3)	5.9

The relaxivity is especially high only
in the substituted aryl compounds, 10, 11 and 12,
i.e., Gd(HAA-DO3A), Gd(HAS-DO3A) and Gd(AAA-DO3A).
One or two hydroxy groups alone do not enhance
relaxivity, as can be seen from L = HP-DO3A, PG-DO3A.
Alkyl or aryl substituents only slightly enhance
relaxivity, as seen from MA-DO3A and PA-DO3A. Both
alkyl and hydroxyalkyl substituents on the aromatic
are effective at enhancing relaxivity (the
hydroxyalkyls are preferred for their increased water
solubility).

Example 4

10-[2-[Methyl[3,5-bis[[(2-methylbutyl)amino]-
carbonyl]phenyl]amino]-2-oxoethyl]-1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid,
monogadolinium salt

A.   N,N'-bis(2-Methylbutyl)-5-[[(phenylmethoxy)-
        carbonyl]-amino]-1,3-benzenedicarboxamide

To a cooled solution of compound A from
Example 3 (15.4 g, 46 mmol) in anhydrous DMA (75 ml)
at 0°C was added benzyl chloroformate (9.4 g, 55.2
mmol). The clear solution was stirred at 0°C for 2
hours. DMA was removed in vacuo. The residue was
dissolved in EtOAc (150 ml), and was washed with
aqueous NaHCO$_3$ solution (30 ml) and with H$_2$O (2 x 50
ml). The organic layer was dried over anhydrous
MgSO$_4$ and the solvent removed to obtain the crude
product as an oily liquid. Recrystallization of the
crude material from EtOAc/hexanes afforded the title
A product as a white solid (17.0 g), m.p. 130.5-
132.5°C.
Elemental analysis calc'd for C$_{26}$H$_{35}$N$_3$O$_4$:
        C, 68.85; H, 7.78; N, 9.26; O, 14.11%.
Found: C, 68.64; H, 7.91; N, 9.20%.

B.   N,N'-bis(2-Methylbutyl)-5-[methyl[(phenyl-
        methoxy)carbonyl]amino]-1,3-benzenedi-
        carboxamide

To a suspension of NaH (0.58 g, 24.2 mmol) in
anhydrous THF (25 ml) was added a solution of the
title A compound (10.0 g, 22 mmol) in anhydrous THF
(60 ml). MeI (15.7 g, 110 mmol) was added and the
reaction mixture was stirred at room temperature for

1 hour. THF was removed in vacuo. The solid was
dissolved in EtOAc and washed with H_2O and then with
aqueous NaCl solution. The EtOAc layer was dried
over anhydrous $MgSO_4$ and the solvent removed to
5 obtain the title B compound.
Elemental analysis calc'd for $C_{27}H_{37}N_3O_4$:
C, 69.35; H, 7.98; N, 8.99; O, 13.69%.
Found: C, 69.10; H, 8.03; N, 8.91%.

10 C.   5-(Methylamino)-N,N'-bis(2-methylbutyl)-1,3-
benzenedicarboxamide
To a solution of the title B compound (13 g,
27.8 mmol) in MeOH (50 ml) was added 1,4-cyclo-
hexadiene (20 ml) and 10% Pd/C (3.25 g). The mixture
15 was refluxed for 0.5 hour. The solid was filtered
through a celite cake and the solvent was removed to
obtain the crude product. Recrystallization from hot
EtOAc afforded the title C product as white crystals
(5.2 g), m.p. 160.1-160.8°C.
20 Elemental analysis calc'd for $C_{19}H_{31}N_3O_2$:
C, 68.43; H, 9.37; N, 12.60; O, 19:60%.
Found: C, 68.13; H, 9.50; N, 12.57%.

D.   5-[(Chloroacetyl)methylamino]-N,N'-bis(2-
25   methylbutyl)-1,3-benzenedicarboxamide
To a solution of the title C compound (5.2 g,
15.6 mmol) in anhydrous DMA (150 ml) was added
chloroacetyl chloride (2.43 g, 5.9 mmol). The
solution was stirred at room temperature for 1.5
30 hours. The mixture was cooled. Water (20 ml) was
added and the solvent removed in vacuo. The residue
was dissolved in EtOAc and washed with aqueous $NaHCO_3$
solution, then with water. The organic layer was
dried over anhydrous $MgSO_4$ and the solvent removed to

obtain the crude product. Recrystallization from hot EtOAc afforded the title D compound as white crystals (6.0 g), m.p. 170.0-171.5°C.

Elemental analysis calc'd for $C_{21}H_{32}N_3O_3Cl$:

5   C, 61.53; H, 7.87; N, 10.25; Cl, 8.65;

O, 11.71%.

Found: C, 61.77; H, 7.83; N, 10.39; Cl, 8.41%.

E.   10-[2-Methyl[3,5-bis[[(2-methylbutyl)amino]-

10   carbonyl]phenyl]amino]-2-oxoethyl]-1,4,7-10-
tetraazacyclododecane-1,4,7-triacetic acid

DO3A sulfate (4.35 g, 9.8 mmol) was dissolved in H_2O (100 ml) and the pH of the solution adjusted to 9.8 by adding 10 N NaOH. To this solution at 85°C

15 was added a solution of the title D compound (5.7 g, 13.9 mmol) in EtOH (100 ml) over a period of 45 minutes. The pH was maintained at 9.8 by adding 5 N NaOH. The mixture was heated at 85°C for 44 hours. The solvents were removed _in vacuo_. The solid was

20 dissolved in H_2O (300 ml) and EtOAc (100 ml) and the cloudy solution was stirred at 85°C for 2 hours until the mixture turned clear. The two layers were separated. The aqueous layer (pH 7) which contained the crude product was applied to a 300 ml column of

25 CHP-20 resin using EtOH/H_2O (0-10%) as an eluent. The fractions containing the desired compound were combined and removal of the solvent afforded the title E product as a monosodium salt (2.9 g).

Elemental analysis calc'd for C, 54.86; H, 7.68; N,

30   13.17; O, 19..76%

Found: C, 54.84; H, 8.09; N, 12.73%.

F.   10-[2-[Methyl[[3,5-bis[[(2-methylbutyl)amino]-
carbonyl]phenyl[amino]-2-oxoethyl]-1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid,
<u>monogadolinium salt</u>

5   The title E compound (700 mg, 0.97 mmol) was
dissolved in H2O (8 ml) and the pH of the solution
adjusted to 4.5 by adding diluted HOAc. To this
solution was added a solution of Gd(OAc)$_3$·4H$_2$O (1.21
g, 1.3 mmol) in H$_2$O (10 ml). The mixture was stirred

10   at 45°C for 24 hours. The solution was then applied
to a 600 ml column of CHP-20 resin, using EtOH/H$_2$O
(0-50%) as an eluent. The fractions containing the
desired compound were combined and removal of the
solvent afforded 770 mg of the title product.

15   Elemental analysis calc'd for C$_{34}$H$_{52}$N$_7$O$_9$Gd·1.10H$_2$O:

          C, 46.42; H, 6.21; N, 11.14; Gd, 18.28;

          O, 16.74%.

Found: C; 46.68; H, 6.35; N, 10.88%.

20   <u>Example 5</u>

10-[2-[[4-[[2,3-Dihydroxypropyl)amino]carbonyl]-
phenyl]amino]-2-oxoethyl-1,4,7,10-tetraazacyclodo-
<u>decane-1,4,7-triacetic acid, monogadolinium salt</u>

25

A.   N-(2,3-Diacetylpropyl)-4-carboxyamido
<u>nitrobenzene</u>

        To a solution of methyl 4-nitro benzoate (18.1
g, 100 mmol) in 200 ml of MeOH was added 3-amino-1,2-

30   propanediol (18.2 g, 200 mmol) and the mixture was
refluxed for 24 hours. The product then was directly
acetylated. Methanol was removed <u>in vacuo</u>. The
residue was dissolved in 100 ml of pyridine and 80 ml
of acetic anhydride was added. The solution was

stirred at room temperature for 24 hours. The solution was cooled and water was added to decompose the excess acetic anhydride. The solvents were removed *in vacuo*. The residue was dissolved in EtOAc

5   and it was washed with H_2O, 10% HCl and finally with brine. The organic layer was dried and removal of the solvent afforded 28.3 g of the title A compound as a yellowish solid (87.3 mmol), m.p. 101.5-102.8°. Elemental analysis calc'd for $C_{14}H_{16}N_2O_7$:

10   C, 51.85; H, 4.97; N, 8.64;
Found: C, 51.69; H, 5.00; N, 8.58.

B.   N-(2,3-Diacetylpropyl)-4-carboxyamido aniline

A solution of the title A compound (12 g, 37

15   mmol) in 120 ml of EtOAc was mixed with 5% Pd/C (1.2 g). The solution was hydrogenated at 45 psi pressure until the pressure dropped down to a constant value. The solid was then filtered. The filtrate was concentrated to dryness and 10.8 g of the title B

20   product as a foaming liquid was obtained (36.7 mmol). TLC: Silica gel, R_f 0.70, EtOAc, visualized by UV.

C.   4[(Chloroacetyl)amino]-N-(2,3-dihydroxy-
         propyl)-1-benzenecarboxamide

25   To a cooled solution of the title B compound (9.3 g, 31.6 mmol) in 120 ml of anhydrous DMA was added chloroactyl chloride (5.3 g, 46.9 mmol). The solution was stirred at room temperature for 1 hour. The mixture was cooled, 20 ml of saturated aqueous

30   $NaHCO_3$ solution added and the mixture was concentrated *in vacuo*. The residue was dissolved in EtOAc and the organic layer washed with H_2O and brine. The organic layer was dried over anhydrous $MgSO_4$ and evaporated to dryness. To deprotect the

acetate groups, the residue was dissolved in 130 ml
of MeOH. To this solution, a solution of 230 mg Na
in 5 ml MeOH was added. It was stirred at room
temperature for 1 hour. Dowex 50 (H$^+$ form) was added
until pH 7. The resin was filtered and the solution
was concentrated to a volumeof 50 mL.
Crystallization of the product gave 6.2 g of solid
title C compound (21.6 mmol), m.p. 184.6-185.5°C.
Elemental analysis calc'd for $C_{12}H_{15}N_2O_4Cl$:

C, 50.40; H, 5.39; N, 9.48; Cl, 12.00;

Found: C, 50.78; H, 5.28; N, 9.59; Cl, 12.19.

D.   10-[2-[[4-[[(2,3-Dihydroxypropyl)amino]-
carbonyl]phenyl]amino]-2-oxoethyl]-1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid

DO3A sulfate (6.0 g, 13.5 mmol) was dissolved
in 200 ml of H_2O and the pH of the solution was
adjusted to 9.8 by adding 10 N NaOH. To this
solution at 85°C was added a solution of the title C
compound (5.8 g, 20.2 mmol) in 200 ml of EtOH over a
period of 45 minutes. The pH was maintained at 9.8
by adding 5 N NaOH. As the reaction proceeded, the
mixture turned clear. The mixture was heated at 85°C
for 26 hours. The solvents were removed in vacuo.
The crude material was dissolved in 500 ml of H2O and
applied to a 2-liter column ofanion exchange resins.
The column was eluted with a gradient of Et_3NH^+⁻HCO3
buffer, 5 mM to 200 mM. The fractions containing the
desired compound were combined and concentrated in
vacuo. The title D compound (6.1 g) was obtained as
the mono triethylammonium salt (8.8 mmol).
Elemental analysis calc'd for $C_{32}H_{55}N_7O_{10}$ · 0.29H2O:

C, 54.67; H, 7.97; N, 13.95;

Found: C, 54.71; H, 8.14; N, 13.94.

E.   10-[2-[[4-[[(2,3-Dihydroxypropyl)amino]-
        carbonyl]phenyl]amino]-2-oxoethyl]-1,4,7,10-
        tetraazacyclododecane-1,4,7-triacetic acid,
        monogadolinium salt

5   700 mg (1.0 mmol) of the title D compound
(mono triethylammoniun salt) was dissolved in 10 ml
of H₂O and the pH of the solution adjusted to 4.5 by
adding diluted HOAc. To this solution was added a
solution of Gd(OAc)₃·4H₂O (540.4 mg, 1.3 mmol) in 15
10   ml of H₂O. The mixture was stirred at 45°C for 24
hours. The solution was then diluted and applied to
a column of CHP-20 resin. The column was eluted with
H₂O, then with increasing amount of EtOH (5-20%).
Evaporation of the combined fractions containing the
15   desired product afforded 300 mg of the pure title
compound (0.40 mmol).
Elemental analysis calc'd for C₂₆H₃₇N₇O₁₀Gd·0.82H₂O:
        C, 40.79; H, 5.09; N, 10.98;
Found: C, 40.81; H, 5.14; N, 10.91.

20

Example 6

10-[N-(4-Nitrophenyl)acetamido]-1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid,
25   monogadolinium salt

A.   10-[N-(4-Nitrophenyl)acetamido]-1,4,7,10-
        tetraazacyclododecane-1,4,7-triacetic acid

30   A solution of 2-chloro-4'-nitroacetanilide (3
g, 14 mmol) in DMSO (30 ml) was slowly added into a
solution of DO3A (5.8 g, 16.8 mmol) in water (30 ml)
whose pH was adjusted to 10 by the addition of 10 N
NaOH at 50°C. The reaction was maintained at 50°-60°C

and the pH was kept at 10 for 54 hours. The yellow
precipitate was filtered and dissolved in water (150
ml). The pH of the resulting solution was adjusted
to ca.2 by the addition of 1.0 N HCl. The resulting

5 solution was then applied to a column of CHP-20P
resin. The column was eluted with water (3L),
followed by 5% (1L), 10% (1L) and 20% (1.5L) EtOH
containing water in the order maintained. The
fractions containing the desired compound were

10 combined and concentrated _in vacuo_ to give the yellow
title A product (2.6 g).
Analysis calc'd for $C_{22}H_{32}N_6O_9$ 1.30 H_2O:
          C, 48.23; H, 6.36; N, 15.34; O, 30.07%.
Found: C, 47.94; H, 6.48; N, 15.72; H_2O, 4.26%.

15

B.   10-[N-(4 Nitrophenyl)acetamido]-1,4,7,10-
          tetraazacyclododecane-1,4,7-triacetic acid,
          monogadolinium salt   ,
          The title A free acid (580 mg, 1.114 mmol)

20 suspended in water (5 mL) was treated with gadolinium
acetate (602 mg, 1.48 mmol, 1.33 eq.) in water (3.5
mL) at 65°C. Upon mixing the starting materials the
solution became homogeneous but after 25 minutes a
pale yellow solid precipitated out; Filtration and

25 washing of the solid with water gave the title
product (470 mg).
Analysis calc'd for $C_{22}H_{29}N_6O_9Gd\cdot0.69$ H_2O:
          C, 38.23; H, 4.43; N, 12.16; O, 22.4...
Found: C, 38.34; H, 4.48; N, 12.09; H_2O, 1.80%.

30

Example 7

10-[N-(4-Aminophenyl)acetamido]-1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid,
5 monogadolinium salt

A.   10-[N-(4-Aminophenyl)acetamido]-1,4,7,10-
         tetraazacyclododecane-1,4,7-triacetic acid,
         monotriethylammonium salt

10   To a solution of compound A of Example 6 (5.3
g, 10.1 mmol) in water (150 ml) whose pH was adjusted
to 7.0 by the addition of 10 N NaOH was added 10%
Pd/C catalyst (2.17 g, 1.0 mmol of Pd). The solution
was hydrogenated at room temperature under a hydrogen
15 atmosphere (20-25 psi) for 3 hours. The reaction
mixture was then filtered to remove the catalyst.
The filtrate was concentrated and applied on a 5 x 20
cm column of anion exchange resin. The column was
eluted with a step gradient (5 mM-10 mM) of aqueous
20 triethylammonium bicarbonate solution. The fractions
containing the desired compound were combined and
concentrated to yield 4.2 g of the title A mono-
triethylammonium salt.
Analysis calc'd for $C_{28}H_{49}N_7O_7.1.91$ $H_2O.0.34$ $NCC_2H_5I_3$
25   C, 54.29; H, 8.78; N, 15.47;), 21.45%.
Found: C, 53-92; H, 9.18; N, 15-58; H_2O, 5-45% (H-NMR
spectrum supports the presence of 0.34 mol-equivalent
of $N(C_2H5)_3$).

B.   10-[N-(4-Aminophenyl)acetamido]-1,4,7,10-
30   tetraazacyclododecane-1,4,7-triacetic acid,
         monogadolinium salt
         To a solution of the Compound B of example 6
(3.39 g, 5 mmol) in MeOH (95 ml) and water (18 ml)
was added 10% Pd/C catalyst (1.06 g, 0.5 mmol of Pd).

The solution was hydrogenated at room temperature under hydrogen atmosphere (20-25 psi) for 10 hours. The solution containing the catalyst was then filtered. After the filtrate was evaporated to dryness, the residue was crystallized from MeOH (30 ml) to give the product (3.04 g).
Analysis calc'd for $C_{22}H_{31}N_6O_7Gd \cdot 4.18$ H_2O:

C, 36.49; H, 5.48; N, 11.61; 0,24.70%.
Found: C, 36.22; H, 5.41; N, 11.41; H_2O, 10.4%.

Example 8

10-[[N-{4-(N'-Isothiocyanto)phenyl]acetamido]]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, monogadolinium salt

This activated species was prepared in situ as follows:

To an aqueous solution (7 ml) of the compound of Example 7.B (194.7 mg, 0.3 mmol) was added thiophosgene (138 mg, 1.2 mmol) in $CHCl_3$ (6 ml). The biphasic mixture was stirred at room temperature until the starting material was consumed completely. The aqueous layer was separated, and its pH (1.3) was adjusted to 5.9 by the addition of 1.0 N NaOH. Mass spectral analysis of the solution showed the presence of a peak at m/e 692 corresponding to Example 8. and

Example 9

10-[N-[4-(N'-Methylthioureido)phenyl]acetamido]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, monogadolinium salt

To a solution of the title compound from
Example 7 (194.7 mg, 0.3 mmol) in H_2O (7.5 ml) was
added a solution of thiophosgene (138 mg, 1.2 mmol)
in $CHCl_3$ (6 ml). The biphasic mixture was stirred at
5   room temperature until the compound was consumed
completely. The aqueous layer (pH 1.0-1.5) was
separated, and the $CHCl_3$ layer was washed with water
(1 ml x 2). The combined aqueous layers were treated
with 1 N NaOH to adjust the pH of the so-formed title
10   8 solution to 6.0. Methylamine (18.04 mg, 0.58 mmol)
was then added, and the reaction mixture was stirred
for 10 minutes. The resulting solution was loaded on
a CHP-20 column and eluted with water and ethanol.
The desired title 9 compound was eluted out by 10% of
15   ethanol to give the desired product (129 mg).
Analysis calc'd for $C_{24}H_{34}N_7O_7SGd \cdot 2.99$ H_2O:

C, 37.16; H, 5.19; N, 12.64; O, 20.60%.
Found: C, 37.00; H, 5.16; N, 12.39; H_2O, 6.94%.

20   Example 10

10-[N-[4-(N',N'-Diethylaminothioureido)phenyl]-
acetamido]-1,4,7,10-tetraazacyclododecane-1,4,7-
triacetic acid, monogadolinium salt

25

To a solution of the title gadolinium chelate
of Example 7 (324 mg, 0.5 mmol) in H_2O (15 ml) was
added a solution of thiophosgene (230 mg, 2 mmol) in
$CHCl_3$ (10 ml). The biphasic mixture was stirred at
30   room temperature until the chelate was consumed
completely to provide a solution of the Example 8
isothiocyanato product. The aqueous layer (pH 1.0-
1.5) was separated, and the $CHCl_3$ layer was washed
with water (2 ml x 2). The combined aqueous layers

were treated with 1 N NaOH to adjust the pH of the isothiocyanto solution to 6.0. Diethylamine (73.1 mg, 1.0 mmol) was then added, and the reaction mixture was stirred for 10 minutes. The resulting

5 solution was loaded on a CHP 20P column and eluted with water and aqueous ethanol. The desired compound was eluted out by 10% ethanol to give the desired product (286 mg).

Analysis calc'd for $C_{27}H_{40}N_7O_7SGd$ 2.31 H_2O:

10   C, 40.26; H, 5.58; N, 12.17; O,18.49%.

Found: C, 40.30; H, 5.71; N, 11.99; H_2O, 5.16%.

Example 11

15 10,10'[[[[[(1,2-Ethanediyl)diimino]bis(thioxomethyl)-diimino]bis(4,1-phenylene)]diiminobis[1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid], gadolinium (1:2) salt

20   The Example 8 isothiocyanato derivative solution was prepared as in Examples 8, 9 and 10. Ethylenediamine (11.2 mg, 0.19 mmol) dissolved in water (1.0 mL) was added to this solution. The pH of the resulting mixture was initially increased to

25 10.04 and then decreased to 7.88 at the end of 3 hours stirring. Concentrated ammonium hydroxide was used to quench the excess Example 8 chelate. The crude product, obtained after removal of the water and ammonium hydroxide, was purified by CHP 20P

30 chromatography. The desired product was eluted out by 10% ethanol to give the dimeric gadolinium chelate (150 mG).

Analysis calc'd for $C_{48}H_{66}N_{14}O_{14}S_2Gd_2 \cdot 2.19$ H_2O:

     C, 38.92; H, 4.79; N, 13.24; O, 4.49; S, 4.33%.

Found: C, 39.07; H, 4.77; N, 13.19; S, 3.95;
H₂O, 2.66%

Example 12

10,10'-[[[[(Thioxomethyl)bis(imino)bis(4,1-
phenylene)]bis(imino)]bis(2-oxo-2,1-ethanediyl)]-
1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid,
gadolinium (1:2) salt

The product of Example 7 (194.7 mg, 0.3 mmol)
dissolved in water (0.5 ml) was added to the Example
8 isothocyanato solution described above. The
reaction mixture was stirred at room temperature for
10 hours. The resulting solution was applied to a
CHP 20P column. The column was eluted with H₂O, 2%,
4% and 6% of EtOH containing water in the order
mentioned. The desired compound was eluted by 6% of
EtOH to give the title product (259 mg).
Analysis calc'd for C₄₅H₆₀N₁₂O₁₄SGd₂·4.37H₂O:
C, 38.11; H, 4.88; N, 11.85; O, 20.72, S,
2.2b%.
Found: C, 38.35; H, 5.03; N, 11.80; H₂O, 5.55%.

Example 13

10,10',10''-[[[[[[Iminobis(2,1-ethanediyl)triimino]-
tris(thioxomethyl)]-triimino]tris-(4,1-phenylene)]-
triimino]tris(2-oxo-2,1-ethanediyl)]tris[1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid],
gadolinium (1:3) salt

Tris(2-aminoethyl)amine (19.7 mg, 0.135 mmol)
dissolved in water (0.5 mL) was added to the Example

8 isothiocyanato solution described above. The pH of
the resulting mixture read 10.10 upon mixing, and
then decreased to 7.88 after stirring 18 hours.
Concentrated ammonium hydroxide was added to quench
5 the excess Example 8 compound. The crude product,
which was obtained after removal of the water and
ammonium hydroxide, was purified by CHP 20P
chromatography. The desired product was eluted by
20% ethanol to give the trimeric gadolinium chelate
10 (230 mG).

Analysis calc'd for $C_{75}H_{105}N_{22}O_{21}S_3Gd_3 \cdot 6.92H_2O$:

C, 38.44; H, 5.11; N, 13.15; O, 19.0b; S, 4.10%.

Found: C, 38.75; H, 5.09; N, 13.14; S, 3.76;

H_2O, 5.32%

15

Example 14

10-[2-[[2-(4-Nitrophenyl)ethyl]amino]-2-oxoethyl]-
1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid,
20 monogadolinium salt

A.   2-Chloro-N-[2-(4-nitrophenyl)ethyl]acetamide

To a solution of p-nitrophenethylamine
(hydrochloride salt, 6.0 g, 29.7 mmol) in anhydrous
25 DMA (50 ml) and Et_3N (3.0 g, 29.7 mmol) was added
chloroacetyl chloride (6.71 g, 59.4 mmol). The
reaction mixture was stirred at room temperature for
2 hours. The solvent was removed in vacuo, the
residue was dissolved in EtOAc and the solution was
30 washed with aqueous $NaHCO_3$ and brine. The organic
layer was dried and the solvent removed to obtain the
crude product as a yellow solid. Recrystallization
of this material from hot EtOAc/ hexanes (10:1)
afforded the anilide as a white crystal (5.5 g).

Elemental analysis calc'd $C_{10}H_{11}N_2ClO_3$:

C, 49.50; H, 4.57; N, 11.54; Cl, 14.61;
O, 19.78%.

Found: C, 49.74; H, 4.50; N, 11.12; Cl, 14.35%.

B.   10-[2-[[2-(4-Nitrophenyl)ethyl]amino]-2-
oxoethyl-1,4,7,10-tetraazacyclododecane-1,4,7-
triacetic acid

DO3A sulfate (6.0 g, 13.5 mmol) was dissolved
in H_2O (100 ml) and the pH of the solution was
adjusted to 9.5 by adding 10 N NaOH. To this
solution at 80°C was added a solution of the title A
compound (5.5 g, 22.7 mmol) in EtOH (80 ml) over a
period of 30 minutes. The pH was maintained at 9.5
by adding 5 N NaOH. The mixture was heated at 80°C
for 48 hours, and the solvents were removed in vacuo.
The solid was dissolved in H_2O and washed twice with
EtOAc. The aqueous layer was evaporated by a water
pump at 40°C to remove traces of EtOAc. The solution
was diluted to 600 ml and applied to a 1.5-liter
column of anion exchange resin. The column was
eluted with a step gradient of aqueous $Et_3NH^{+-}HCO_3$
solution, 5 mM to 200 mM. The fractions containing
the desired compound were combined and concentrated
in vacuo. The title B compound (7.67 g) was obtained
as the mono triethylammonium salt.

C.   10-[2-[[2-(4-Nitrophenyl)ethyl]amino]-2-
oxoethyl]-1,4,7,10-tetraazacyclododecane-
1,4,7-triacetic acid, monogadolinium salt

The title B compound (monotriethylammonium
salt, 65.3 mg, 0.1 mmol) was dissolved in H_2O (5 ml)
and the pH of the solution adjusted to 4.5 by adding
diluted HOAc. To this solution was added a solution

of Gd(OAc)$_3$·4H$_2$O (52.8 mg, 0.13 mmol) in H$_2$O (5 ml).
The mixture was stirred at room temperature for 1
hour. The solution was then applied to a 400 ml
column of CHP-20 resin, using EtOH/H$_2$O (0-15%) as an
5 eluent. The fractions containing the desired
compound were combined and removal of the solvent
afforded the title compound as a monogadolinium salt
(450 mg).
Elemental analysis calc'd for C$_{24}$H$_{33}$N$_6$GdO$_9$·1.93 H$_2$O:
10   C, 38.88; H, 5.01; N, 11.33; Gd, 21.21;
        O, 23.57%.
Found: C, 38.81; H, 5.15; N, 11.40%.

Example 15

15

10-[2-[[3,5-bis[[(2-hydroxyethyl)amino]-
carbonyl]phenyl]amino]-2-oxoethyl]-1,4,7,10-
tetraazacyclododecane-1,4,7-triacetic acid-gadolinium
(III) complex

20

A.   N,N'-bis(2-hydroxyethyl)-5-nitro-1,3-
        benzenedicarboxamide
        A solution containing dimethyl-5-
nitroisophthalate (23.9 g, 100 mmol) and ethanolamine
25 (13.4 g, 220 mmol) in methanol (300 mL) was refluxed
for 48 hours. The solvent was removed by evaporation
under reduced pressure. The crude product was
purified by crystallization from EtOAC:MeOH (_.1 v/v)
to afford 20.0 g of pure N,N'-bis(2-hydroxyethyl)-5-
30 nitro-1,3-benzenedicarboxamide as white crystals.
m.p.= 151.3-151.4°C, uncorrected
Elemenatal Analysis: Calculated for C$_{12}$H$_{15}$O$_6$N$_3$·0.8
        H2O: C, 48.25; H, 5.12; N, 14.07; O, 32.57%

B.   N,N'-bis(2-acetoxyethyl)-5-amino-1,3-
        benzenedicarboxamide

        A solution containing N,N'-bis(2-
hydroxyethyl)-5-amino-1,3-benzenedicarboxamide (6.0
5   g, 20 mmol) and acetic anhydride (11 mL) in pyridine
     (50 mL) was stirred at room temperature for 4 hours.
     Water (1 mL) was added to decompose the excess
     anhydride and the solvent was removed _in vacuo_.   The
     residue was dissolved in EtOAc (50 mL) and the
10   solution was successively washed with H_2O (50 mL),
     10% HCl (50 mL), and saturated NaCl solution (50 mL).
     The aqueous layer was reextracted with EtOAc (50 mL)
     and the organic layer was combined with the previous
     extract.   The EtOAc layer was dried over $MgSO_4$,
15   filtered, and evaporated to dryness.   The residue
     (7.3 g, 19 mmol) was dissolved in hot MeOH (100 mL)
     and hydrogenated using cat. 10% Pd/C for 3 hours at
     50 psi H_2.   The filtrate was evaporated to dryness
     and afforded 6.4 g of pure title B product.
20   Elemental Analysis Calculated for: $C_{16}H_{21}O_6N_3$:
             C,54.70; H, 6.02; N, 11.96; O, 27.32%.
     Found: C, 54.97; H, 6.19; N, 12.10; ROI, 0.12; H_2O,
     0.000%.

25   C.   N,N'-bis(2-acetyloxy)ethyl]-5-[(chloroacetyl)-
             amino-1,3-benzenedicarboxamide

             Chloroacetyl chloride (3 mL) was added
     dropwise to an ice-cold solution containing N,N'-
     bis(2-acetoxyethyl)-5-amino-1,3-benzenedicarboxamide
30   (6.4 g, 18 mmol) in anhydrous DMA (5 mL).   After one
     hour, the reaction mixture was neutralized by the
     addition of saturated aqueous $NaHCO_3$ and the solution
     was concentrated in vacuo to a syrup.   The syrup was
     dissolved in EtOAc (100 mL) and was successively

washed with H_2O (2 x 50 mL) and brine (50 mL). The
organic layer was dried over $MgSO_4$, filtered and
evaporated to yield 6.2 g of pure title C product.

5   Elemental Analysis calculated for $C_{18}H_{22}O_7N_3Cl \cdot 0.52$
           $H_2O \cdot 0.55$ CD_3OD: C, 48.75; H, 4.85; N, 9.19;
           Cl, 7.75; O, 24.50%.
       Found: C,48.35; H, 5.04; N, 9.30; Cl, 8.20; ROI,
           0.00; H_2O), 2.16% (desorption KF).

10

       D.   5-[chloroacetyl)amino]-N,N'-bis(2-hydroxy-
               ethyl)-5-amino-1,3-benzenedicarboxamide
               A solution of N,N'-bis[2-(acetyloxy)ethyl]-5-
       [(chloroacetyl)amino-1,3-benzenedicarboxamide (6.2 g,
15   14 mmol) in MeOH (20 mL) was treated with NaOMe (600
       mg, 10.5 mmol). After 2 hours at room temperature,
       the mixture was neutralized with AG-50W-X2 (H+ form)
       resin. The resin was removed by filtration and the
       solution was evaporated to dryness to afford 4.8 g of
20   crude material. An analytical sample of the title D
       product was crystallized from MeOH.
       m.p. = 178.4-180.6°C, uncorrected.
       Elemental Analysis calcd. for $C_{14}H_{18}O_5N_3Cl \cdot 0.16$ H_2O:
           C, 48.51; H, 5.33; N, 12.12; Cl, 10.23; O,
25   23.81%
       Found: C, 48.73; H, 5.45; N, 12.16; Cl, 10.27; ROI,
           0.09; H_2O, 0.83 (desorption KF).

       E.   10-[2-[[3,5-bis[[(2-hydroxyethyl)amino]-
30   carbonyl]phenyl]amino]-2-oxoethyl]-1,4,7,10-
               tetraazacyclododecane-1,4,7-triacetic acid,
               monosodium salt.
               A suspension of 5-[chloroacetyl)amino]-N, N'-
       bis(2-hydroxyethyl)-1,3-benzenedicarboxamide (1.0 g,

2.9 mmol) in water (20 mL) was added to a basic
solution (pH 9.8) of DO3A (692 mg, 2.0 mmol) in water
(3 mL) maintained at 80°C. A small amount of ethanol
(5 mL) was added to during the course of the addition
5   to aid in the solubilization of the chloroanilide.
During the course of the reaction, the pH was
maintained constant by occasional addition of small
aliquots of 10N NaOH. The progress of the reaction
was monitored by HPLC. After 20 hours, the reaction
10   was neutralized to pH 6.75 with 1N HCl and applied to
a CHP-20 column (150 mL). The product was eluted
with water. Fractions containing pure product were
combined and evaporated to dryness to afford 1.1 g of
the title E product as a white glassy material.
15   m.p.= 176°C (decomp), uncorrected.
Elemental Analysis calc'd for $C_{28}H_{42}O_{11}N_7Na \cdot 1.97$ H_2O:
         C, 47.30; H, 6.51; N, 13.79; Na, 3.23; O,
         29.17;
Found: C, 47.34; H, 6.75; N, 13.45; Na, 2.91; ROI,
20   not determined; H_2O, 4.98% (desorption KF).

F.   10-[2-[[3,5-bis[[(2-hydroxyethyl)amino]-
         carbonyl]phenyl]amino]-2-oxoethyl]-1,4,7,10-
         tetraazacyclododecane-1,4,7-triacetic acid,
25   monogadolinium salt (VII).
         A solution containing the title E ligand (200
mg, 306 μmol) in water (100 μL) was adjusted to pH
4.69 by addition of HOAc. A solution of Gd(OAc)$_3 \cdot 4$
H_2O (187 mg, 461 μmol) in water (50 μL) was added to
30   the ligand and the reaction was stirred for 17 hours
at 80°C. The crude product was applied directly onto
a CHP-20 column (100 mL). After washing the column
with water, the pure product was eluted with 5% EtOH
in water. Fractions containing the product were

pooled and evaporated to yield 160 mg of pure
product.

MS (positive FAB, m/z): (M + H)$^+$ at 809 (^{158}Gd).

Elemental Analysis: calc'd for $C_{28}H_{38}O_{11}N_7Gd\cdot2.72$
H_2O: C, 39.34; H, 5.12; N, 11.4; Gd, 18.39;
O, 25.68%.

Found: C, 39.41; H, 5.52; N, 11.19; ROI, 17.85; H_2O,
5.74% (desorption KF).

Example 16

10,10',10'',10''',10'''',10''''''-[[[[[[[(Nitrilo-
tri-2,1-ethanediyl)tris(nitrilo)]hexakis-(2,1-ethane-
diyl)]hexakis(imino)hexakis-(carbonothioyl)]hexakis-
(imino)]hexakis-(4,1-phenylene)]hexakis-(imino)]-
hexakis-(2-oxo-2,1-ethanediyl)]hexakis[1,4,7,10-tetra-
azacyclododecane-1,4,7-triacetic acid], gadolinium
__(1:6) salt__

A.   Tris[bis(2-p-toluenesulfonylaminoethyl)-
        __aminoethyl]amine__

        Tris(2-aminoethyl)amine (0.73 g, 4.96 mmol)
and N-tosyl aziridine (5.9 g, 30.0 mmol) were added
in 5% aqueous EtOH (10 ml). The reaction mixture was
stirred at room temperature. Over a period of 2
hours a white precipitate formed, which made stirring
difficult. After another portion of 5% aqueous EtOH
(10 ml) was added, the reaction mixture was stirred
for 21 hours. The solid was filtered and washed with
cold ethanol to obtain the crude product (6.3 g).
The crude product was recrystallized twice from hot
acetonitrile (120 ml each) to afford the title

product, tris[bis(2-p-toluenesulfonylaminoethyl)-
aminoethyl]amine (3.4 g) in 52% yield.

HPLC: Retention time 7.7 minutes; YMC C_8 basic 120Å,
5   4.6 x 250 mm; 48% acetonitrile in 25 mM HCl; UV at
254 nm; flow rate, 1 ml/min. IR (KBr): 3285, 1599,
1325, 1157, 1094 and 552 cm^{-1}. MS(FAB): m/z: 1329.5
(M+H)$^+$; 904 [M-CH$_2$N(CH$_2$CH$_2$NHTs)$_2$]; 438
[CH$_2$CH$_2$N(CH$_2$CH$_2$NHTs)$_2$]; 424 [CH$_2$N(CH$_2$CH$_2$NHTs)$_2$].
10   ^{1}H-NMR (DMSO): δ 2.35 (s, 18H, CH$_3$); 2.31, 2.65 (t,
36H, CH$_2$); 7.32 - 7.67 (m, 24H, benzene ring).
^{13}C-NMR (DMSO): 22.05 (CH$_3$); 54.38 (CH$_2$); 127.57,
130.70, 138.75, 143.61 (benzene ring). Anal. Calcd.
for C$_{60}$H$_{84}$N$_{10}$O$_{12}$S$_6$ • 0.18 H$_2$O: C, 54.06; H, 6.38; N,
15   10.51, S, 14.43, Found: C, 53.95; H, 6.37; N, 10.55;
S, 14.29. H$_2$O, 0.25% (desorption Karl-Fisher).

B.   <u>Tris[bis(2-aminoethyl)aminoethyl]amine</u>
    Tris[bis(2-p-toluenesulfonylaminoethyl)-
20   aminoethyl]amine (1 g, 0.75 mmol) was treated with
concentrated H$_2$SO$_4$ (5 ml) under a N$_2$ atmosphere at
130°C for 48 hours. After cooling the reaction
mixture to 0°C, diethyl ether (40 ml) was added in
small portions maintaining the temperature under
25   10°C. The resulting hygroscopic precipitate was
filtered and dissolved in H$_2$O (5 ml). The solution
was basified to pH 13 with 10 N NaOH and extracted
with CH$_2$Cl$_2$. The CH$_2$Cl$_2$ layer was then dried over
Na$_2$SO$_4$ and concentrated to obtain the title product,
30   tris[bis(2-aminoethyl)aminoethyl]amine (90 mg).
^{1}H-NMR(D$_2$O): 2.47 - 2.68 (t, 36H, CH$_2$). ^{13}C-NMR(D$_2$O):
37.51 (CH$_2$NH$_2$); 50.71 (NCH$_2$CH$_2$NH$_2$); 55.91 (NCH$_2$CH$_2$N).
MS(FAB): m/z: 427 (M+NA)$^+$; 405 (M+H)$^+$; 288
[MCH$_2$N(CH$_2$CH$_2$NH$_2$)$_2$]$^+$; 276 [MCH$_2$CH$_2$N)CH$_2$CH$_2$NH$_2$)$_2$]$^+$.

C.   10,10',10'',10''',10'''',10'''''-[[[[[[[(Nitrilo-
tri-2,1-ethanediyl)tris(nitrilo)]hexakis-(2,1-
ethanediyl)]hexakis(imino)hexakis-(carbono-
thioyl)]hexakis-(imino)]hexakis-(4,1-phenyl-
ene)]hexakis-(imino)]hexakis-(2-oxo-2,1-
ethanediyl)]hexakis[1,4,7,10-tetraazacyclo-
dodecane-1,4,7-triacetic acid], gadolinium
(1:6) salt

Tris[bis(2-aminoethyl)aminoethyl]amine (50 mg,
0.124 mmol) dissolved in water (1.8 ml) was added to
Gd(IPA-DO3A) which was prepared as described in the
following paragraph (and is also described as the
title compound of Example 8 above). The reaction
mixture was stirred at room temperature for 20 hours.
The reaction mixture was then concentrated to
dryness. The residue was dissolved in H_2O (5 ml),
and the pH of the resulting solution adjusted to 11.9
by the addition of 1M NaOH. The resulting aqueous
solution was applied to a CHP 20 P column (2.5 x 20
cm). The column was eluted with water and water
containing 2%, 5%, 10%, 20% and 25% EtOH in the
sequence indicated. The desired compound was eluted
out by 25% of EtOH to afford the title compound C
(234 mg) as an off-white solid in 46% yield.
$C_{156}H_{222}N_{46}O_{42}S_6$ • 6 Gd• 23.49 H_2O MW 3606.14 FW
4972.82.
HPLC: Retention time 7.9 minutes; AMP-303 ODS 200°A,
4.6 x 250 mm; 16% acetonitrile in 50 mM tris and 10mM
EDTA (pH 7.0); UV at 254 nm; flow rate, 1 ml/min. IR
(KBr): 3437, 1618, 1508, 1385, 1317 and 1084 cm^{-1}.
MS: m/z: 4550.6 $(M+H)^+$; 2227.7 $(M+2H)^{+2}$. Anal.
Calcd. for $C_{156}H_{222}N_{46}O_{42}S_6Gd_6$ • 23.49 H_2O: C, 37.68;
H, 5.45; N, 12.96, S, 3.87, Found: C, 38.02; H, 5.70;

N, 12.83; S, 3.53. H2O, 8.51% (desorption
Karl-Fisher).

In situ preparation of Gd(IPA-DO3A): To an aqueous
5   solution (22 ml) of monogadolinium salt of 10-[N-
(4-aminophenyl)acetamido]-1,4,7,10-tetraazacyclo-
dodecane-1,4,7-tri-acetic acid, Gd(APA-DO3A) (603.3
mg, 0.93 mmol) was added a solution of thiophosgene
(427.7 mg, 3.72 mmol) in CHCl3 (18 ml). The biphasic
10 mixture was stirred at room temperature and the
progress of the reaction monitored by HPLC using a
Nucleosil C18 column. When the conversion to the
expected product was completed, the aqueous layer was
separated, and its pH (1.0) adjusted to 8 by the
15 addition of 1.0 N NaOH.

Abbreviations used herein:

DMA = dimethylacetamide
20 THF = tetrahydrofuran
MeI = methyl iodide
EtOAc = ethyl acetate
MeOH = methanol
EtOH = ethanol
25 HOAc = acetic acid
Ac = acetyl
DMSO = dimethylsulfoxide
NaOMe = sodium methoxide